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HUMAN RECEPTOR FOR INTERLEUKIN-12

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by resting and activated T- and NK-cells (M.K bayashi et al, supra; S. H. Chan et al, J. Exp. Med. 173,869,1991), and promotion of T_h1-type helper cell responses (R. Manetti et al, J. Exp. Med. 177,1199,1993; C.-S. Hsieh et al, Sci nce 260,547,1993)..

The biological activity of IL-12 is mediated by the binding of the IL-12 molecules to cell surface, or plasma membrane, receptors on activated T- and NK cells; however, the contributions of the individual subunits, p35 and p40, to receptor binding and signal transduction remain unknown. Studies with labeled IL-12 have shown that this binding occurs in a specific and saturable manner (R.Chizzonite et al, J. Immunology 148,3117,1992). The IL-12 receptor on activated T-cells shows 3 classes of affinities: high = 5-20 pM, intermediate = 50-200 pM and low = 2-6 nM. Affinity crosslinking of labeled ligand to these cells shows the receptor to consist of a protein of about 150 Kd size. It is not clear at this point whether the biologically functional IL-12 receptor consists of one or more subunits.

Using a non-neutralizing monoclonal antibody to the IL-12 receptor, we have now succeeded in isolating a human cDNA that encodes a low affinity (5-10 nM) IL-12 receptor. This protein belongs to the cytokine receptor superfamily and within that family shows strongest homology to gp130.

In order for a molecule such as IL-12 to exert its effect on cells, it is now accepted by those skilled in the art that the molecule must interact with molecules, located on cell membranes, referred to as

receptors. Patents which exemplify disclosures of interleukin receptors include Honjo et al., U.S. Pat. N . 4,816,565; and Urdal et al., U.S. Pat. No. 4,578,335, th discl sures of which are incorporated by reference. Fanslow, et al., Science 248:739-41 (May 11, 1990) presented data showing that the effect of IL-1 in vivo could be regulated via the administration of a soluble form of its receptor. The last paragraph of the Fanslow paper, the disclosure of which is incorporated by reference, describes the types of therapeutic efficacy that administration of soluble IL-1 receptor is expected to have. What types of therapeutic efficacy that administration of soluble IL-12 receptor is expected to have can also be contemplated therefor by those skilled in the art.

The availability of the purified receptor, in soluble form, presents therapeutic possibilities as well. Addition of soluble IL-12 receptor interferes with the effect of the interleukin on the cells, since the molecule cannot bind to the cell membrane as freely. Hence, an aspect of the invention is the treatment of pathological conditions caused by excess activity of cells possessing IL-12 receptors by adding an amount of soluble IL-12 receptors sufficient to inhibit binding of IL-12 to the aforementioned cells. This methodology can also be modified, and the soluble receptor can be used as a screening agent for pharmaceuticals. Briefly, a pharmaceutical which works as an IL-12 antagonist can Jo so by blocking the binding of IL-12 to the IL-12 receptor. Prior to determining whether a material would be effective in vivo, one may use the purified IL-12 receptor in connection with a potential pharmaceutical to determine if there is binding. If not, then the

pharmaceutical may no longer be a desirable candidate. If there is in fact binding, further testing may be indicated.

SUMMARY OF THE INVENTION

The present invention is directed towards an isolated cDNA encoding a human low affinity IL-12 receptor protein or subunit thereof. When expressed in mammalian cells, the cDNA gives rise to substantially homogeneous IL-12 receptor protein that binds IL-12 in a specific and saturable manner with an apparent affinity of about 5 - 10 nM.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: DNA sequence of human IL-12 receptor cDNA clone No. 5. (translated portion = nucleotides 65 to 2050) (SEQ ID NO:1).

Figure 2: Amino acid sequence of human IL-12 receptor protein as deduced from cDNA sequence of Figure 1. (boxed N-terminal sequence = proposed signal sequence; boxed area AA No. 541 to 571 = transmembrane area; 6 potential N-linked glycosylation sites in the extracellular portion are also boxed) (SEQ ID NO:2).

Figure 3A: Scatchard analysis of IL-12 binding to recombinant human IL-12 receptor expressed in COS cells.

Figure 3B: Scatchard analysis of 2-4E6 antibody binding to rec mbinant human IL-12 receptor expressed in COS cells.

Eigure 4: Analysis of the size of human recombinant IL-12 receptor expressed in COS cells. COS cells transfected with human IL-12 receptor cDNA were labeled and lysed as described herein. Human IL-12 receptor protein was immunoprecipitated and the products were analyzed on a 4 - 20 % gradient gel under reducing conditions. 5 µg of each listed antibody were used. They were Control IgG3 = isotype-matched negative control antibody; 2-4E6 = anti-human IL-12 receptor antibody; 4D6 = anti human IL-12 negative control antibody. Sizes of marker proteins are indicated in KDa on left.

Figure 5: Analysis of the size of human IL-12 receptor RNA transcripts. RNA blotting was performed as described herein. Poly A+ RNA from the following cell sources were used: Peripheral blood mononuclear cells without PHA induction = PBMC - PHA; PBMC + PHA; PHA induction for 3 days; Kit225/K6 = human T-cell line constitutively expressing IL-12 receptor. RNA size markers in kilobases (kb) are shwon on the left. Two human IL-12 receptor RNA transcripts of about 3.5 kb and 2.8 kb are evident. They probably represent differentially spliced RNAs.

Figure 6: Inhibition of ^{125}I-IL-12 Binding to IL-12 (IL-12R) Receptor by Mouse Anti-IL-12R Antiserum

Figure 7: Characterization of the IL-12 Binding Proteins on IL-12R Positive Human cells by Affinity-Crosslinking

Figure 8: Immunoprecipitation of the Solubilized ^{125}I-IL-12/IL-12R Crosslinked Complex by Anti-IL-12R Antibodies

Figure 9: Equilibrium binding of ^{125}I-2-4E6 to PHA-activated PBMC at Room Temperature

Figure 10: Equilibrium Binding of ^{125}I-2-4E6 to Human K6 Cells at Room Temperature

Figure 11: Inhibition of ^{125}I-2-4E Binding to K6 Cells by Purified 2-4E6 (24E6), Human IL-12 (HUIL-12) and Control Antibody (Control IgG)

Figure 12: Equilibrium Binding of ^{125}I-IL-12 to Human K6 Cells at Room Temperature

Figure 13: Equilibrium Binding of ^{125}I-IL-12 to Detergent Solubilized IL-12R from K6 Cells

Figure 14: Western Blot Analysis of Detergent Solubilized IL-12R with mAb 2-4E6

Figure 15: Detection of IL-12 Receptor on Human Cells by Flow Cytometry

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed towards an isolated cDNA (SEQ ID NO:1) that encodes a human low-affinity IL-12 receptor protein (SEQ ID NO:2) or subunit thereof. The amino acid sequence of the substantially homogeneous IL-12 receptor protein as deduced from the cDNA sequence is shown in Figure 2.

The IL-12 receptor cDNA is useful for the following purposes:

Expression of recombinant IL-12 receptor protein in high levels and its use as an antigen allows production of additional neutralizing monoclonal and polyclonal antibodies. Such neutralizing antibodies can be used in in vivo model settings to elucidate the role that IL-12 and its receptor play in normal as well as pathologic immune responses (i.e. disease states that are aggravated by activated T- and NK-cells like autoimmune diseases, graft versus host disease and rheumatoid arthritis).

IL-12 receptor proteins can be administered, for example, for the purpose of suppressing immune responses in a human. A variety of diseases or conditions are caused by an immune response to alloantigen, including allograft rejection and graft-versus-host reaction. In alloantigen-induced immune responses, IL-12 receptor may suppress lymphoproliferation and inflammation which result upon activation of T cells. IL-12 receptor may therefore be used to effectively suppress

alloantigen-induced immune responses in the clinical treatment of, for example, rejection of allografts (such as skin, kidney, and heart transplants), and graft-versus-host reactions in patients who have received bone marrow transplants.

IL-12 receptor may also be used in clinical treatment of autoimmune dysfunctions, such a rheumatoid arthritis, diabetes and multiple sclerosis, which are dependent upon the activation of T cells against antigens not recognized as being indigenous to the host. IL-12 receptor may also be useful in treatment of septic shock in which interferon gamma produced in response to IL-12 plays a central role in causing morbidity and mortality (G.M. Doherty et al., 1992, J. Immunol. 149:1666).

Purified IL-12 receptor compositions will be useful in diagnostic assays for IL-12 or IL-12 receptor, and also in raising antibodies to IL-12 receptor for use in diagnosis or therapy. In addition, purified IL-12 receptor compositions may be used directly in therapy to bind or scavenge IL-12, thereby providing a means for regulating the immune or inflammatory activities of IL-12. In its use to prevent or reverse pathologic immune responses, soluble IL-12 receptor can be combined with other cytokine antagonists such as antibodies to the IL-2 receptor, soluble TNF receptor, the IL-1 receptor antagonist, and the like.

The dose ranges for the administration of the IL-12 receptor proteins and fragments thereof may be determined by those of ordinary skill in the art without undue experimentation. In general,

appropriate dosages are those which are large enough to produce the desired effect, for example, blocking the binding of endogenous IL-12 to its natural receptor. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. The IL-12 receptor proteins and fragments thereof can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, anti-microbials, anti-oxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th Ed., Mack Eds., 1980.

As used herein, "DNA sequence" refers to a DNA polymer, in the form of a separate fragment or as a component of a larger DNA construct, which has been derived from DNA isolated at least once in substantially pure form, i.e., free of c ntaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the sequence and its component nucleotide sequences by standard biochemical methods, for example, using a cloning vector. Such sequences are preferably provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. However, it will be evident that genomic DNA containing the relevant sequences could also be used. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

As used herein, "recombinant expression vector" refers to a plasmid comprising a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Structural elements intended for use in various eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue.

This residue may optionally be subsequently cleaved from the expressed recombinant protein to pr vide a final product.

MATERIALS AND METHODS

Proteins and Plasmids

Recombinant human IL-12 (U. Gubler et al, Proc. Natl. Acad. Sci.(USA) 88:4143, 1991) and murine IL-12 (D. Schoenhaut et al, J. Immunology 148:3433, 1992) were obtained as described therein.

The murine anti human IL-12 receptor monoclonal antibody 2-4E6 used herein was generated as described herein below in Examples 1 to 16 and was purified from ascites fluids by affinity chromatography on protein G-agarose according to the manufacturer's instructions (Genex). The proteins were labeled with I-125 by a modification of the Iodogen method as described (Pierce Chemical Co., Rockford, IL). Radiospecific activities of 5000 - 7000 cpm/fmole for IL-12 and 1500 - 2500 cpm/fmole for the 2-4E6 antibody were typically obtained.

The plasmid pEF-BOS was obtained from Dr. Nagata at the Osaka Bioscience Institute in Japan. The plasmid is based on a pUC 119 backbone and contains the elongation factor 1 alpha promoter to drive expression of genes inserted at the BstXI site (S. Mizushima and S. Nagata, Nucl. Acids Res., 1990, 18:5322).

The murine anti human IL-12 receptor monoclonal antibody 2-4E6 was prepared, characterized, and generated as follows:

Example 1

Preparation, Characterization & Purification of Hybridoma Antibodies

Balb/c mice (Charles River Laboratories) were immunized by the intraperitoneal route with PHA(phytohemagglutanin)-activated human PBMC (PHA-activated PBMC) at 6 x 10^7 cells/mouse. Mice received 5 subsequent booster injections of between 2-5 x 10^7 cells over a six month period. For preparation of activated spleen cells, 2 mice were injected intraperitoneally and intravenously with 1 x 10^7 and 2.5 x 10^6 cells, respectively, on two successive days, starting four days prior to the cell fusion. Spleen cells were isolated from these mice and fused with SP2/0 cells at a ratio of 1:1 with 35% v/v polyethylene glycol 4000 (E. Merck) according to the method of Fazekas et al., J. Immunol. Methods 35, 1 (1980). The fused cells were plated at a density of 6 x 10^5 cells/ml/well in 48-well cluster dishes in IMDM supplemented with 10% FBS, glutamine (2 mM), β-mercaptoethanol (0.1 mM), gentamicin (50 g/ml), 5% ORIGEN hybridoma cloning factor (IGEN, Inc.), 5% P388D1 supernatant (Nordan, R.P., et al., J. Immunol., 139:813 (1987)) and 100 Units/ml rHuIL-6. Hybridoma supernatants were assayed for specific anti-IL-12 receptor antibodies by: 1) immunoprecipitation of the soluble complex of ^{125}I-HuIL-12 crosslinked to IL-12 receptor (^{125}I-IL-12/IL-12R), 2) inhibition of ^{125}I-HuIL-12 binding to PHA-activated PBMC's, and 3) differential binding to IL-12 receptor positive cells versus receptor negative cells). Hybridoma cell lines secreting specific anti-receptor antibodies were cloned by limiting dilution. Antibodies

were purified from ascites fluids by affinity chromatography on Protein G bound to cross-linked agarose according to the manufacturer's protocol (Genex).

Example 2

Preparation of Human PHA Lymphoblasts and IL-12 Receptor Binding Assays

Human peripheral blood mononuclear cells were isolated (see Gately et al, J. Natl. Cancer Inst. 69, 1245 (1982)) and cultured at 37°C at a density of 5 x 105 cells/ml in tissue culture medium (TCM) containing 0.1% PHA-P (Difco). After 3 days, the cultures were split 1:1 with fresh TCM, and human rIL-2 was added to each culture to give a final concentration of 50 units/ml. The cultures were then incubated for an additional 1-2 days, prior to use in assays.

PHA-activated human PBMC were washed once in binding buffer (RPMI-1640, 5% FBS, 25 mM HEPES pH 7.4) and resuspended in binding buffer to a cell density of 7 X 10^6 cells/ml. Lymphoblasts (7 X 10^5 cells) were incubated with various concentrations of ^{125}I-IL-12 (5-10000 pM) at room temperature for the designated times. Cell bound radioactivity was separated from free ^{125}I-IL-12 by centrifugation of the assay mixture through 0.1 ml of an oil mixture (1:2 mixture of Thomas Silicone Fluid 6428-R15 : A.H. Thomas, and Silicone Oil AR 200 : Gallard-Schlessinger) at 4°C for 90 sec at 10,000 x g. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter. Non-specific binding was determined by inclusion of 100 nM unlabeled IL-12 in the assay. Incubations were carried out

in duplicate or triplicate. Receptor binding data wer analyzed by using the non-linear regression programs EBDA and LIGAND as adapted for the IBM personal computer by McPherson, J. Pharmacol Methods 14, 213 (1985) from Elsevier-BIOSOFT.

Example 3

Affinity Cross-Linking of ^{125}I-IL-12 to IL-12 Receptor Bearing Cell Lines

IL-12 receptor bearing cells were incubated with ^{125}I-IL-12 (100-500 pM) in the presence or absence of excess unlabeled IL-12 for 2 hr at room temperature. The cells were then washed with ice-cold PBS pH 8.3 (25mM Sodium Phosphate pH 8.3, 0.15 M NaCl and 1 mM $MgCl_2$) and resuspended at a concentration of 0.5-1.0x10^7 cells/ml in PBS pH 8.3. BS3 (Pierce) in dimethyl sulfoxide was added to a final concentration of 0.4 mM. Incubation was continued for 30 min. at 4°C with constant agitation. The cells were washed with ice-cold 25 mM Tris-HCl (pH 7.5), 0.15 m NaCl and 5 mM EDTA and then solublized at 0.5 - 1.0 x 10^8 cells/ml in solubilization buffer (50 mM Tris-HCl (pH 8.0) containing 8 mM CHAPS, 0.25 M NaCl, 5 mM EDTA, 40 µg/ml PMSF, 0.05% NaN_3, and 1% BSA) for 1 hr at 4°C. The extracts were centrifuged at 12,000 x g for 45 min. at 4°C to remove nuclei and other debris.

Example 4

Immunoprecipitation Assay of the Soluble Complex of ^{125}I-IL-12 Crosslinked to Human IL-12R.

For the immunoprecipitation assay, hybridoma culture supernatant (0.5 ml), diluted antisera, or purified IgG was added to a microfuge tube containing 0.1 ml of a 50% suspension of either goat-anti-mouse IgG coupled to agarose (SIGMA CHEM. CO.) or Protein G coupled to Sepharose 4B (Pharmacia). The assay volume was brought up to 1.0 ml with IP buffer (8 mM CHAPS in PBS (.25 M NaCl), 1% BSA, & 5 mM EDTA) and the mixture was incubated on a rotating mixer for 2 hr at room temperature. The beads were pelleted by centrifugation, resuspended in 1 ml IP buffer containing ^{125}I-IL-12/IL-12R (10-20,000 cpm) and the mixture was incubated on a rotating mixer for 16 hr at 4°C. After this incubation, the beads were pelleted by centrifugation and washed twice in IP buffer without BSA. The ^{125}I-labeled receptor complex bound to the solid phase antibodies was released by adding 100 µl of 2x Laemmli sample buffer (Nature 227, 680 (1970)) with and without 10% β-mercaptoethanol and heating for 5 min. at 95°C. The immunoprecipitated proteins were analyzed by SDS-PAGE on 8% or 4-15% gradient polyacrylamide gels and visualized by autoradiography.

Example 5

Assays for IL-12R Solubilized from Cells Expressing IL-12 Receptor.

To confirm that the antibodies identified by the immunoprecipitation assay were specific for IL-12R, an immunoprecipitation/soluble IL-12R binding assay was developed. As described in Example 1 above, antibodies (as hybridoma supernatant,

purified IgG (50 µg) or antisera) were immobilized by binding to goat anti-mouse IgG c upled to agarose (100 µl; Sigma Chemical Co.) or protein G coupled t Sepharose 4B (100 µl; Pharmacia). For some experiments, antibodies were covalently crosslinked to protein G-Sepharose 4B, before being used in the assay. See Stern and Podlaski, Techniques in Protein Chemistry (1993). The immobilized antibodies were resuspended in IP buffer (0.3 ml) and 0.2 ml of a detergent solubilized extract of PHA-activated PBMCs or K6 cells that contained IL-12R was added. To prepare the detergent solubilized IL-12R preparation, the cells were washed with ice-cold 25 mM Tris-HCl (pH 7.5), 0.15 M NaCl and 5 mM EDTA and then solublized at 1.5 x 10^8 cells/ml in solubilization buffer (50 mM Tris-HCl, pH 8.0, containing 8mM CHAPS, 0.25 M NaCl, 5 mM EDTA, 40 µg/ml PMSF, 0.05% NaN_3, and 1% BSA) for 1 hr at 4°C. The extracts were centrifuged at 120,000 x g for 60 min. at 4°C to remove nuclei and other debris. The mixture was incubated on a rotating mixer for 16 hr at 4°C. After this incubation, the beads were pelleted by centrifugation and resuspended in IP buffer (0.15 ml) containing ^{125}I-HuIL-12 at concentrations ranging from 0.05 to 7.5 nM. The IL-12R immobilized on the antibody coated beads was incubated with ^{125}I-HuIL-12 for 2 hrs. at room temperature on a shaker. Following this incubation, the beads were pelleted, washed twice with IP buffer and the bound radioactivity determined in a gamma counter. Nonspecific binding was determined by inclusion of 70 nM unlabeled human IL-12 in the assay. Solubilized IL-12R binding data were analyzed according to the method of Scatchard, (Assn. N.Y. Acad. Sci. 51, 660 (1949)) by using the nonlinear regression programs

EBDA and Ligand as adapted for the IBM PC by McPherson, supra from Elsevier-BIOSOFT.

Example 6

Competitive Inhibition of ^{125}I-IL-12 Receptor Binding by Antibodies

The ability of hybridoma supernatant solutions, purified IgG, or antisera to inhibit the binding of ^{125}I-IL-12 to PHA-activated lymphoblasts was measured as follows: serial dilutions of culture supernatants, purified IgG or antisera were mixed with activated lymphoblasts (1-1.5 x 10^6 cells) in binding buffer (RPMI-1640, 5% FBS + 25 mM HEPES pH 7.4) and incubated on an orbital shaker for 1 hour at room temperature. ^{125}I-HuIL-12 (1 x 10^5 cpm) was added to each tube and incubated for 1-2 hours at room temperature. Non-specific binding was determined by inclusion of 10 nM unlabeled IL-12 in the assay. Incubations were carried out in duplicate or triplicate. Cell bound radioactivity was separated from free ^{125}I-IL-12 by centrifugation of the assay through 0.1 ml of an oil mixture as described above. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter.

Example 7

Labeling of Human IL-12 and Mab 2-4E6 with ^{125}I

Human IL-12 and purified 2-4E6 IgG were labeled with ^{125}I by a modification of the Iodogen method (Pierce Chemical Co., Rockford, IL).

Iodogen was dissolved in chloroform and 0.05 mg dried in a 12 x 15 mm borosilicat glass tube. For radiolabeling, 1.0 mCi Na[^{125}I] (Amersham, Chicago, IL) was added to an Iodogen-coated tube containing 0.05 ml of Tris-iodination buffer (25 mM Tris-HCL pH 7.5, 0.4 M NaCl and 1 mM EDTA) and incubated for 4 min at room temperature. The activated ^{125}I solution was transferred to a tube containing 0.05 to 0.1 ml IL-12 (7 µg) or IgG (100 µg) in Tris-iodination buffer and the reaction was incubated for 9 min at room temperature. At the end of the incubation, 0.05 ml of Iodogen stop buffer (10 mg/ml tyrosine 10% glycerol in Dulbecco's PBS, pH 7.40) was added and reacted for 3 min. The mixture was then diluted with 1.0 ml Tris-iodination buffer, and applied to a Bio-Gel P10DG desalting column (BioRad Laboratories) for chromatography. The column was eluted with Tris-iodination buffer, and fractions (1 ml) containing the peak amounts of labeled protein were combined and diluted to 1 x 108 cpm/ml with 1% BSA in Tris-iodination buffer. The TCA preciptable radioactivity (10% TCA final concentration) was typically in excess of 95% of the total radioactivity. The radiospecific activity was typically ~ 1500 to 2500 cmp/fmol for 2-4E6 IgG and 5000 to 7000 cpm/fmole for IL-12.

Example 8

Binding Assays of ^{125}I-2-4E6 to Intact Cells

PHA-activated human PBMC were washed once in binding buffer (RPMI 1640, 5% FBS and 25 mM HEPES, pH 7.4) and resuspended in binding buffer to a cell density of 1.5 x 10^7 cells/ml. Lymphoblasts (1.5 x 10^6 cells) were incubated with various concentrations of ^{125}I-2-4E6-

IgG (.005 to 2 nM) at room temperature for 1.5 hrs. Cell bound radioactivity was separated from free ^{125}I-2-4E6 IgG by centrifugation f the assay mixture through 0.1 ml silicone il at 4°C for 90 seconds at 10,000 x g. The tip containing the cell pellet was excised, and cell bound radioactivity was determined in a gamma counter. Non-specific binding was determined by inclusion of 67 nM unlabeled 2-4E6 IgG in the assay. Incubations were carried out in duplicate or triplicate. Receptor binding data were analyzed by using the nonlinear regression programs EBDA, Ligand and Kinetics as adapted for the IBM personal computer by McPherson, supra from Elsevier BIOSOFT.

Example 9

Expression of Recombinant IL-12R in COS Cells and Determination f ^{125}I-2-4E6 Binding

COS cells (4-5 X 10^7) were transfected by electroporation with 25 µg of plasmid DNA expressing recombinant human IL-12R, as describe hereinbelow, in a BioRad Gene Pulser (250 µF, 250 volts) according to the manufacturer's protocol. The cells were plated in a 600 cm^2 culture plate, harvested after 72 hours by scraping, washed and resuspended in binding buffer. Transfected cells (8 x 10^4 were incubated with increasing concentrations of ^{125}I-labeled 2-4E6 or IL-12 at room temperature for 2 hrs. Cell bound radioactivity was separated from free ^{125}I-labeled 2-4E6 or IL-12 as described above.

Example 10

Western Blot Analysis of Soluble IL-12R with mAb 2-4E6

PHA-activated PBMC were washed 3 times with ice-cold PBS and solubilized at 0.5 - 1 x 10^8 cells/ml in solubilization buffer (50 mM Tris-HCl pH 8.0 containing 8 mM CHAPS, 0.25 M NaCl, 5 mM EDTA, 40 µg/ml PMSF, .05% NaN_3 and 1 mg/ml BSA) for 1 hr at 4°C. The extracts were centrifuged at 12,000 x g for 45 min. at 4°C to remove nuclei and other debris. The extracts were incubated with 2-4E6 IgG or control IgG bound to goat-anti-mouse IgG immobilized on cross-linked agarose (Sigma Chemical Co.). The precipitated proteins were released by treatment with 0.1 M glycine pH 2.3, neutralized with 3M Tris, mixed with 1/5 volume of 5 x Laemmli sample buffer, and separated by

SDS/PAGE on 8% pre-cast acrylamid gels (NOVEX). The separat d proteins were transferred to nitrocellulose membrane (0.2 µM) for 16 hours at 100 volts in 10 mM TRIS-HCL (pH 8.3), 76.8 mM glycine, 20% methanol and 0.01% SDS. The nitrocellulose membrane was block d with BLOTTO (5.0% w/v nonfat dry milk in PBS + .05% Tween 20) and duplicate blots were probed with ^{125}I-2-4E6 IgG (1 x 106 cpm/ml in 8 mM CHAPS in PBS, 0.25 M NaCl, 10% BSA and 5 mM EDTA) + unlabeled 2-4E6 IgG (67 nM).

Example 11

Analysis of IL-12 Receptor Expression on Human Cells by Fluorescence Activated Cell Sorting with mAb 2-4E6

To stain cells expressing IL-12 receptor, 1 x 10^6 cells in 100 µl staining buffer (PBS containing 2% FBS and 0.1% NaN_3) were incubated with 10 µl of 2-4E6 ascites fluid for 25 min. at 4°C. Cells were then washed twice with staining buffer followed by incubation with a 1:100 dilution of goat F(ab)2 anti mouse Ig-PE (Tago, Burlingame CA) for 25 min. at 4°C. The stained cells were washed twice with staining buffer and then analyzed on a FACScan flow cytometer (Becton Dickinson).

Example 12

Inhibition of IL-12 Binding to Human PHA-Lymphoblasts by Mouse Anti-IL-12R Antiserum.

Mice immunized with PHA-activated PBMCs developed an immune response against the human IL-12R as determined by inhibition of ^{125}I-IL-12 binding to PHA-activated PBMCs (Figure 6) and

immunoprecipitation of the complex of ^{125}I-IL-12 crosslinked to IL-12R (data not shown). The dilutions for half-maximal inhibition of ^{125}I-IL-12 binding to PHA-activated PBMCs were 1/500 and 1/250 for animals 211-1 and 211-2, respectively (Figure 6). These antisera als neutralized IL-12 biologic activity as measured in a PHA-lymphoblast proliferation assay (data not shown). Spleen cells isolated from these mice were fused with SP2/0 myeloma cells and the resulting hybridomas were initially screened for IL-12R specific antibodies by immunoprecipitation of the ^{125}I-IL-12/IL-12R complex and by inhibition of ^{125}I-IL-12 binding to IL-12R.

For Figure 6, ten fold serial dilutions of mouse anti-IL-12R immune serum (#211-1 and #211-2) and normal mouse serum (NMS) were preincubated with PHA-activated PBMC for 60 min at RT (room temperature) before addition of ^{125}I-IL-12 (100 pM). After addition of ^{125}I-IL-12, the reaction was incubated for 1-2 hrs at RT and the cell bound radioactivity was determined. The data are expressed as the % Inhibition of ^{125}I-IL-12 binding in the presence of the immune serum when compared to the specific binding in the absence of serum.

Example 13

Identification and Characterization of Monoclonal Anti-IL-12R Antibodies.

The immunoprecipitation assay identified 13 hybridomas secreting putative non-neutralizing anti-IL-12R antibodies, whereas the IL-12R binding assay identified 3 putative neutralizing IL-12R

antibodies (Table 1). The immunoprecipitation assay measured the abi''ty of the putative anti-IL-12R antibodies that are imm bilized on a solid phase to capture the solubilized complex of ^{125}I-IL-12/IL-12R. To verify that the radioactivity immunoprecipitated by the immobilized antibody was present in the complex of ^{125}I-IL-12/IL-12R, the immunoprecipitated proteins were solubilized, separated by SDS-PAGE and visualized by autoradiography. The preparations of the ^{125}I-IL-12/IL-12R complexes solubilized from PHA-activated PBMC, Kit-225 and K6 cells were resolved into two major radioactive bands, 210-250 kDa and 75 kDa (Figure 7). The 210-250 kDa and 75 kDa complexes were identified as the ^{125}I-IL-12/IL-12R complex and ^{125}I-IL-12 not complexed with the receptor, respectively (Figure 7). See also Chizzonite et al., J. Immunol. 148, 3117 (1992). The radioactive 75 kDa band visualized from the cell extracts co-migrated with ^{125}I-IL-12, indicating that it represented ^{125}I-IL-12 that bound but was not covalently crosslinked to IL-12R. The 210-250 kDa band was not a covalent crosslinked oligomer of ^{125}I-IL-12 because it is not produced when the crosslinking agent BS3 was added directly to ^{125}I-IL-12 (Figure 7).

Hybridoma cells secreting putative anti-IL-12R antibodies were then cloned by limiting dilution and screened by both the immunoprecipitation and inhibition of binding assays that identify non-neutralizing and neutralizing antibodies, respectively. During this cloning and screening process, hybridoma lines secreting putative neutralizing anti-IL-12R antibodies were not recovered, whereas non-neutralizing antibodies were recovered from both the original

immunoprecipitation and inhibitory positive hybridomas. After this initial identification and cloning, a direct binding assay was used to determine if the n n-neutralizing antibodies only bound to cells expressing IL-12R. This assay demonstrated that the non-neutralizing antibodies could be divided into 2 classes, those that bound only IL-12R positive human cells and those that bound to most human cells (data not shown). Representative antibodies from each class, 2-4E6 and 2C6, respectively, were produced in ascites fluid, purified by protein G affinity chromatography and extensively characterized.

For Figure 7, PHA-activated PBMC (PHA-PBMC), Kit-225 (Kit-225) and K6 (K6) cells (1 x 10^7 cells/ml) were incubated with ^{125}I-IL-12 (100-500 pM) for 2 hrs at room temperature in the absence or presence of 25 nM unlabeled IL-12. Cells were then washed, affinity crosslinked with BS3 (0.4 mM final concentration) and a cell extract prepared as described. The cell extract was precipitated with wheat germ lectin bound to solid supports as described. The precipitated proteins were released by treatment with sample buffer and analyzed by SDS-PAGE and autoradiography on a 8.0% slab gel. The complex of ^{125}I-IL-12 crosslinked to the IL-12 receptor migrates as a single major band of approximately 210-250 kDa. The band migrating at 75 kDa is ^{125}I-IL-12 that was bound but not crosslinked to the IL-12 receptor. ^{125}I-IL-12 (IL-12) and ^{125}I-IL-12 that was treated with the BS3 crosslinker (IL-12/BS3) were electrophoresed in parallel lanes as markers for the migration of the 75 kDa IL-12 heterodimer and for any oligomers of IL-

12 that may form with the BS3 crosslinker. The molecular sizes indicated in the margins were estimated from standards run in parallel lanes. Exposure time was 7 days.

Table 1

INITIAL IDENTIFICATION OF HYBRIDOMAS SECRETING ANTI-IL-12 RECEPTOR ANTIBODIES: SPLENOCYTES FROM MICE #211-1 AND #211-2

HYBRIDOMA/ANTIBODY		I.P. ASSAY[1] (cpm bound)	INHIBITION ASSAY[2]
IL-12R 2C6[3]		1900	-
211-1	1A5	722	-
	4E6	840	-
	5C1	312	+
211-2	3B1	1323	-
	4A3	2172	-
	4D6	804	-
	5D5	877	-
	4A5	509	+
	4C6	456	+
	1D1	1395	-
	5E6	2043	-
	2-4E6	2836	-
Control mAb			-

1 I.P. assay measures the amount of ^{125}I-IL-12/IL-12R complex bound by the immobilized antibody.

2 Inhibition assay measures whether the antibody can inhibit ^{125}I-IL-12 binding to PHA-activated PBMC.

3 IL-12R 2C6 is an antibody that both immunoprecipitates the ^{125}I-IL-12/IL-12R complex and binds to many IL-12R positive and negative human cells. This antibody probably recognizes a component closely associated with the IL-12R.

Example 14

Characteristics of Monoclonal Anti-IL-12R Antibody 2-4E6 Binding to Natural IL-12R

MAb 2-4E6 immunoprecipitates the ^{125}I-IL-12/IL-12R complex solubilized from PHA-activated human lymphoblasts, Kit-225 and K6 cells (Figure 8, data shown for PHA-activated PBMC), but does not block ^{125}I-IL-12 binding to IL-12R expressed on these cells. These data suggested that the 2-4E6 antibody was a non-inhibitory or non-neutralizing anti-IL-12R antibody. To confirm that 2-4E6 was an non-inhibitory antibody specific for the IL-12R, 2-4E6 was labeled with ^{125}I and direct binding assays were performed with IL-12R positive and negative cells. ^{125}I-2-4E6 binds to IL-12R bearing cells with an affinity that ranges from 337 pM to 904 pM and identifies between 1500 and 5000 binding sites per cell (PHA-activated PBMC, Figure 9; K6 cells, Figure 10). IL-12 does not block ^{125}I-2-4E6 from binding to PHA-activated PBMCs and confirms that 2-4E6 is a non-inhibitory/non-neutralizing antibody (Figure 11). ^{125}I-2-4E6 binds to other cells expressing IL-12R, such as Kit 225, and YT cells, but does not bind to IL-12R negative cells (non-activated human PBMC, MRC-5 fibroblasts and HL-60 cells (Table 2).

Equilibrium binding assays have demonstrated that ^{125}I-IL-12 identifies 3 separate binding sites on the surface of PHA-activated PBMCs, Kit-225 and K6 cells (Figure 12, data for K6 cells and Table 2). Analysis of this binding data by the method of Scatchard, supra shows

these affinities are approximately 5-20 pM, 50-200 pM and 2-6 nM, respectively. The total number of ^{125}I-IL-12 binding sites per cell are approximately 1500 to 5000, which is in good agreement with the total number of binding sites identified by ^{125}I-2-4E6 (Table 2). The data also suggests that 2-4E6 recognizes the low affinity (2-5 nM) binding component of the IL-12 receptor in much the same manner that the anti-TAC antibody recognizes the low affinity component (p55 subunit) of the IL-2 receptor.

Since the data indicated that mAb 2-4E6 was a non-neutralizing antibody specific for the IL-12R, the molecular weight and ^{125}I-IL-12 binding characteristics of the protein(s) immunoprecipitated by mAb 2-4E6 from the surface of IL-12R positive cells was investigated. The steady state binding of ^{125}I-IL-12 to proteins immunoprecipitated by immobilized 2-4E6 from solubilized extracts of PHA-activated PBMCs, Kit-225 and K6 cells was saturable and specific (Figure 13, data for extracts from K6 cells). Transformation of the binding data by the method of Scatchard, revealed a single site with an apparent affinity of 188 pM. The proteins immunoprecipitated by 2-4E6 from the cell extracts were resolved by SDS-PAGE, transferred to nitrocellulose membrane and probed with ^{125}I-2-4E6 in a western blot. On the western blot, ^{125}I-2-4E6 binds to an approximately 90 kDa protein, that is only immunoprecipitated by 2-4E6 and not by an anti-IL-12 antibody or a control antibody (Figure 14, data shown for PHA-activated PBMCs). In summary, all the data demonstrated that mAb 2-4E6 bound a protein on the surface of IL-12R positive cells that was approximately 90 kDa and bound ^{125}I-IL-12 in a specific manner.

For Figure 8, s luble complexes of ^{125}I-IL-12/IL-12R were prepared from PHA-activiated human PBMC as detailed herein (see also Figure 7) and immunoprecipitated by immobilized antibodies, 2-4E6, 2C6, 4D6, 20C2 and control. The soluble complexes were also precipitated with wheat germ lectin immobilized on crosslinked agarose. The precipitated proteins were analyzed as described herein and in Figure 7. Antibodies 4D6 and 20C2 are non-neutralizing and neutralizing anti-IL-12 antibodies, respectively. 4D6 immunoprecipitates ^{125}I-IL-12/IL-12R complex and free ^{125}I-IL-12, whereas 20C2 only immunoprecipitates free ^{125}I-IL-12. Both 2-4E6 and 2C6 recognize the ^{125}I-IL-12/IL-12R complex. ^{125}I-IL-12 (IL-12) and ^{125}I-IL-12 that was treated with the BS3 crosslinked (IL-12/BS3) were electrophoresed in parallel lanes as markers for the migration of the 75 kDa IL-12 heterodimer and for any oligomers of IL-12 that may form with the BS3 crosslinker. The molecular sizes indicated in the margins were estimated from standards run in parallel lanes. Exposure time was 7 days.

For Figure 9, Lymphoblasts (1 x 10^6 cells) were incubated for 2 hrs at room temperature with increasing concentrations of ^{125}I-2-4E6 in the absence (○) or presence (•) of 25 nM unlabeled 2-4E6. Total (○) and non-specific (•) cell bound radioactivity were determined as described. Specific binding of ^{125}I-2-4E6 (▼) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of

Scatchard as determined by Ligand computer program with a single-site model.

For Figure 10, K6 cells (1 x 10^6 cells) were incubated for 2 hrs at room temperature with increasing concentrations of ^{125}I-2-4E6 in the absence (•) or presence (∇) of 25 nM unlabeled 2-4E6. Total (•) and non-specific (∇) cell bound radioactivity were determined as described. Specific binding of ^{125}I-2-4E6 (▼) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard as determined by Ligand with a single-site model.

For Figure 11, The data are expressed as the amount of ^{125}I-2-4E6 bound [CPM BOUND (Percent)] to the cells in the presence of the indicated concentrations of unlabeled antibody or IL-12 when compared with the total specific binding in the absence of unlabeled competitor.

For Figure 12, K6 cells (1 x 10^6 cells) were incubated for 2 hrs at room temperature with increasing concentrations of ^{125}I-IL-12 in the absence (○) or presence (•) of 50 nM unlabeled IL-12. Total (○) and non-specific (•) cell bound radioactivity were determined as described. Specific binding of ^{125}I-IL-12 (∇) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard as determined by Ligand with a single-site model.

For Figure 13, K6 cells 1.5 x 10^8 cells/ml) were solubilized with 8 mM CHAPS extracti n buffer and the cell extract (0.2 ml) was immunoprecipitated for 16 hrs at 4°C with mAb 2-4E6 immobilized on goat anti-mouse IgG coupled to agarose as described. Following this incubation, the beads were pelleted, washed and resuspended in IP buffer containing ^{125}I-IL-12 at concentrations ranging from 7 pM to 7.5 nM. The IL-12R immobilized on the 2-4E6 coated beads was incubated with ^{125}I-IL-12 for 2 hrs at RT and IL-12R bound radioactivity was determined in the presence of 50 nM unlabeled IL-12. The right hand panels show analysis of the binding data according to the method of Scatchard as determined by Ligand with a single-site model.

For Figure 14, PHA-activated PBMC (1 x 10^8 cells/ml) were solubilized with 8 mM CHAPS extraction buffer and the cell extract (1 ml) was immunoprecipitated as described in Figure 13. Following this incubation, the beads were pelleted, washed and the bound proteins released by treatment with 0.1 M glycine pH 2.3. The released proteins were separated by non-reducing SDS/PAGE on 8% gels transferred to nitrocellulose membrane and probed with ^{125}I-2-4E6 as described. The molecular sizes indicated in the margins were estimated from molecular weight standards (Amersham Prestained High Molecular Weight Standards) run in parallel lanes. Exposure time was 7 days.

TABLE 2
COMPARISON OF THE BINDING OF IL-12 AND 2-4C6 TO HUMAN CELLS EXPRESSING IL-12 RECEPTOR

CELL TYPE	IL-12 BINDING[1]		2-4E6 BINDING[2]	
	K_D (nM)	Sites/cell	K_D (nM)	Sites/cell
Human Cells				
non-activated human PBMC[3]	none detected		none detected	
PHA-PBMC	0.018	312	0.745	1472-2246
(5-7 days)	0.084	501		
(3 sites)	1.800	1406		
K6 cells	0.016	707	0.489	3116-5259
(3 sites)	0.057	939		
	2.400	4036		
Kit-225	0.023	100	0.594	1950
(3 sites)	0.210	250		
	2.360	755		
YT cells	0.006	24	0.904	4522
(2 sites)	0.109	117		
RAJI cells	none detectable		0.450	561
MRC-5	none detectable		none detectable	
HL-60	none detectable		none detectable	

1 Steady state ^{125}I-IL-12 binding assays. Apparent dissociation constant (K_D) and binding sites per cell have been calculated by transformation of the data by the method of Scatchard.

2 Steady state ^{125}I-2-4E6 binding assays. Data transformed by the method of Scatchard.

3 Human peripheral blood mononuclear cells (PBMC) were activated with PHA as described in the methods (PHA-PBMC).

Example 15

MAb 2-4E6 Binding To Human Recombinant IL-12R Expressed in COS Cells.

The characteristics of the protein bound by mAb 2-4E6 fulfilled standard criterion for an IL-12R and therefore 2-4E6 was used in an expression cloning strategy to isolate a cDNA coding for the human IL-12R. A cDNA coding for the human IL-12R was isolated by this method (U. Gubler and A.O. Chua, unpublished observations). The IL-12R cDNA was engineered in a mammalian cell expression vector, transfected into COS-7 cells and the specificity for binding of ^{125}I-IL-12 and ^{125}I-2-4E6 was determined. Steady state binding of ^{125}I-IL-12 to the rIL-12R expressing COS cells identifies a single binding site with an apparent affinity of 2-3 nM and approximately 150,000 sites/cell (Figure 3A). This low affinity IL-12 binding site corresponds to the low affinity site seen in the binding assays with human cells that naturally express IL-12R. The binding of 125I-2-4E6 to rIL-12R expressed in the COS cells is saturable and specific and identifies approximately 200,000 sites/cell (Figure 3B). COS cells transfected with an unrelated plasmid do not bind either ^{125}I-IL-12 or ^{125}I-2-4E6 (data not shown). These data demonstrated unequivocally that mAb 2-4E6 was specific for the low affinity component of the IL-12R.

For Figure 3A, COS cells were transfected with a plasmid expressing human rIL-12R as described. Three days later, transfected cells (1 x

10^4 cells) were incubated for 2 hrs at room temperature with increasing concentration of ^{125}I-IL-12 in the absence (O) or presence (□) of 50 nM unlabeled IL-12. Total (O) and non-specific (□) cell bound radioactivity were determined as described. Specific binding of ^{125}I-IL-12 (▲) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard as determined by Ligand with a single-site model.

For Figure 3B, COS cells were transfected with a plasmid expressing human rIL-12R as described in Methods. Three days later, transfected cells (1 x 10^4 cells) were incubated for 2 hrs at room temperature with increasing concentrations of ^{125}I-2-4E6 in the absence (O) or presence (□) of 50 nM unlabeled 2-4E6. Total (O) and non-specific (□) cell bound radioactivity were determined as described. Specific binding of ^{125}I-2-4E6 (▲) was calculated by subtracting non-specific binding from total binding. The right hand panel shows analysis of the binding data according to the method of Scatchard as determined by Ligand with a single-site model.

Example 16

Analysis of mAb 2-4E6 Binding to IL-12R Positive Human Cells by Fluorescence Activated Cell Sorting (FACS).

The expression level of IL-12R on human cells could be regulated depending on the activation state of the cells, the cell cycle or the type of environment from which the cells are isolated. Previous data had

dem nstrated that PHA activation f PBMC leads to a gradual rise in IL-12R expression, reaching a maximum at 3-4 days after activation and declining thereafter. Desai et al., J. Immunol., 148:3125 (1992). To investigate the heterogeneity of IL-12R expression on PHA-activated PBMCs, Kit-225 and K6 cells, FACS analysis of IL-12R on these cells was determined with mAb 2-4E6 (Figure 15). The fluorescence intensity of binding of 2-4E6 was specific and indicated that these three cell types expressed approximately equal numbers of IL-12R. Interestingly, the FACS analysis indicated that the cell population was fairly homogenous and did not have one population expressing no or low numbers of IL-12R and a second population that expressed very high numbers of IL-12R.

For Figure 15, Day 4 PHA-activated lymphoblasts, Kit-225 and K6 cells were analyzed for IL-12R expressing cells by the indirect fluorescent antibody-labeling technique described. The figure depicts specific staining for IL-12R obtained in the presence of mAb 2-4E6 (IL-12R) and non-specific staining obtained in the presence of a control antibody specific for IL-1 receptor (anti-Hu IL-1R), a control antibody specific for human IL-12 (4D6 + GART-PE CTRL) and the goat anti-mouse antibody conjugated with PE (GART-PE CTRL).

Cell Culture:

Peripheral blood mononuclear cells (PBMC) were isolated from blood collected from healthy donors. The blood was collected into heparinized syringes, diluted with an equal volume of Hank's balanced salt solution (HBSS) and layered over Ficoll-Hypaque. The tubes were

spun at 2000 rpm for 20 minutes at room temperature. PBMC at the interface were collected and pelleted at 1500 rpm for 10 minutes through a 15 ml cushion of 20% sucrose in PBS. Pelleted PBMC were resuspended in tissue culture medium and washed twice in the same medium (RPMI 1640 plus 5 % serum). Finally, the cells were cultured at 0.5 - 1 x 10^6 cells/ml in tissue culture medium plus 1 ug/ml PHA-P (Difco) for 3 days at 37 degrees C in a 5% CO_2 atmosphere. Cells were split 1:1 in culture medium plus 50 U/ml rhuIL-2 (Roche) to yield >95% T-cells. The next day, these cells were used for assessing their responsiveness to IL-12, for radioligand (IL-12) binding assays and in flow cytometry assays for the detection of IL-12 receptors.

Flow cytometric detection of IL-12 receptors on such 4 day activated PHA blasts was performed as follows: the cells were washed twice in PBS and resuspended at 2 x 10^6 cells/ml in PBS plus 2% fetal calf serum and 0.1% sodium azide. All the subsequent steps were carried out at 4 degrees C. 1 x 10^6 cells were incubated in 1 nM human IL-12 for 40 minutes. The cells were washed in FACS buffer and incubated with about 1 ug of biotinylated rat anti human p40 IL-12 subunit antibody 4D6 for 20 minutes. Cells were washed again and resuspended in 100 µl of a 5 µg/ml streptavidin-phycoerythritin conjugate (Fisher Biotech) for 15 minutes. The cells were then washed again before analysis on a FACSAN flow cytometer (Becton Dickinson).

Extraction and characterization of RNA

PHA activated cells as described above were harvested at day 2-3 and total RNA was extracted using GuanidinIsothiocyanate/Phenol as

described (P. Chomczynski and N. Sacchi, Anal. Biochem., 162:156, 1987). Poly A+ RNA was isolated from the total RNA by one batch adsorption to oligo dT latex beads as described (K. Kuribayashi et al, Nucl. Acids Res. Symposium Series 19:61, 1988). The mass yield of this purification was about 4%.

RNA blots were performed as follows. RNA was fractionated in 1.2 % agarose gels under denaturing conditions in the presence of 2.2.M formaldehyde and subsequently transferred to nitrocellulose as described (Molecular Cloning, a Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbour Laboratory Press 1989). The RNA blots were hybridized (7 x 10^5 cpm/ml, 30 ml) with labeled probe in 5 x SSC (1X SSC = 0.15 M NaCl-0.015 M NaCitrate) - 50 % formamide - 5 x Denhardts solution (1 X Denhardts = 0.02% polyvinylpyrrolidone, 0.02 % Ficoll 400, 0.02% bovine serum albumin fraction V) - 0.3 % SDS - 250 ug/ml denatured salmon sperm carrier DNA at 37^0 C overnight. The probe was generated by random-primer labeling gel-isolated insert from IL-12 receptor cDNA clone No.5 as described in the above manual. The blots were first quickly rinsed at room temperature in 2 X SSC , then washed in 0.1 X SSC at 50^0 C for 30 minutes, dried and exposed to Kodak XAR film at -70^0 C for 3 days.

cDNA library

From the above polyA+ RNA, a cDNA library was established in the mammalian expression vector pEF-BOS as follows: 3 ug of polyA+ RNA were reverse transcribed into single stranded cDNA using RNaseH minus reverse transcriptase (GIBCO BRL Life Technologies Inc., P.O.Box

9418, Gaithersburg, MD 20898). Th resulting mRNA-cDNA hybrids were converted into blunt ended doublestranded cDNAs by established procedures (U.Gubler and A.Chua, in: Essential Molecular Biology Volume II, T.A. Brown, edit r, pp. 39-56, IRL Press 1991). BstXI linkers (A. Aruffo and B. Seed, Proc. Natl. Acad. Sci (USA) 84, 8573, 1987) were ligated to the resulting cDNAs and cDNA molecules > 800 base pairs (bp) were selected over a Sephacryl SF500 column. A Sephacryl SF 500 column (0.8 x 29 cm) was packed by gravity in 10 mM Tris-HCl pH 7.8 - 1 mM EDTA - 100 mM NaAcetate. BstXI linkered cDNA was applied to the column and 0.5 ml fractions were collected. A small aliquot of each fraction was run on a 1% agarose gel, the gel was dried down and the size distribution of the radioactive cDNA visualized by exposure of the gel to X-ray film. cDNA molecules larger than 800 bp were selected in this fashion, pooled, concentrated by ethanol precipitation and subsequently ligated to the cloning vector. The cloning vector was the plasmid pEF BOS (see reference supra) that had been cut with BstXI and purified over two consecutive gels. 300 ng of plasmid DNA were ligated to 30 ng of size selected cDNA from above in 60 ul of ligation buffer (50 mM Tris-HCl pH 7.8 - 10 mM $MgCl_2$ - 10 mM DTT - 1 mM rATP - 25 ug/ml bovine serum albumin) at 15^0 C overnight. The following day, the ligation reaction was extracted with phenol, 6 ug of mussel glycogen were added, and the nucleic acids were precipitated by ethanol. The precipitate was dissolved in water and the precipitation was repeated, followed by a wash with 80% ethanol. Finally, the pellet was dissolved in 6 ul of water and 1 ul aliquots were subsequently electroporated into E. Coli strain DH-10B (BRL). By electroporating 5 parallel aliquots in this

fashion, a library of about 10 million rec mbinants was generated f r future use.

Screening for IL-12 receptor cDNAs by panning

The basic principle of the panning method has been described in A. Aruffo and B. Seed, Proc. Natl. Acad. Sci (USA) 84, 8573, 1987 as discussed below. Ten library aliquots each representing about 50,000 clones were plated on LB amp plates and were grown up overnight. The next day, the colonies from each pool were scraped off into a separate 50 ml aliquot of LB + amp and the cultures were grown for another two hours before plasmid DNA was extracted using QIAGEN plasmid kits. The ten separate DNA pools were then transfected into COS cells, using the DEAE dextran technique (2 million COS cells/9 cm diameter plate and 2.5 µg DNA) (Molecular Cloning, a Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbour Laboratory Press 1989). 2 to 3 days later, the COS cells were detached from the plates using 0.5 mM EDTA/0.02% Na Azide in PBS and a single cell suspension was prepared for each pool. The monoclonal anti IL-12 receptor antibody 2-4E6 as discussed above was subsequently bound to the cells in suspension (10 µg/ml in PBS-0.5mM EDTA-0.02% Na Azide-5% FCS, 1 hour, on ice). The cell suspension was then spun through a layer of 2% Ficoll in the above buffer (tabletop centrifuge, 1000 rpm, 4 minutes) to eliminate the excess unbound antibody and the cells were gently resuspended in the same buffer. The cells from one pool were subsequently added to one bacterial petri dish (9cm diameter) that had been coated with polyclonal goat anti mouse IgG (20 µg/ml in 50 mM

Tris-HCl pH 9.5, RT/ON) and blocked with 1% BSA in PBS (37 degree C / 1 hour). COS cells were panned in this way for 2 hours at RT. Nonadhering cells were then gently washed off with PBS and the remaining adherent cells in the dishes lysed by the addition of 0.8 ml of Hirt lysis solution (0.6% SDS-10 mM EDTA). After transferring to Eppendorf tubes, the lysates were made 1 M NaCl, incubated ON at +4 degrees C and then spun at 15,000 rpm for 10 minutes in the cold. The supernatants were extracted with phenol once, 12 µg of mussel glycogen was added and the DNA precipitated twice by adding 0.5 volumes of 7.5 M NH_4OAc and 2.5 volumes of ethanol. The resulting DNA pellet was washed once with 80% ethanol, dried and taken up in 1 µl of distilled H_2O. The entire prep was then electroporated into E. coli strain DH-10B and the resulting colonies grown up ON. This represents one panning cycle. The ten library aliquots were panned each one separately for a total of three cycles.

From the last cycle of each pool, DNA was again extracted and this time transfected into COS cells plated on plastic one-chamber microscopic slides (2 slides per pool). 2-3 days after transfection, to one of the slides was bound labeled human IL-12 (10^6 cpm/ml = 300 pM in RPMI 1640 plus 5% FCS for 2-3 hours at 4 degrees C) and to the other slide labeled monoclonal Ab 2-4E6 (2 x 10^6 cpm/ml = 1 nM in RPMI 1640 plus 5% FCS for 1 hour at RT). The slides were washed in PBS, fixed for 40 seconds in a cold mixture of methanol:acetone (7:3) and air dried. The slides were subsequently dipped in Kodak photographic emulsion NTB2, air dried and exposed in a light-tight container for 2 - 4 days at 4 degrees C. They were developed in Kodak D10 developer

according t the manufacturer's instructions and evaluated under a light microscop using a 10 to 40 fold bright field magnification. One of the ten pools, numb r 5, showed a large number of positive cells both for IL-12 and 2-4E6 binding. E. coli clones from this 3 x panned pool were subsequently picked into a microtiterplate (3 clones per well for a total of 288 clones). Pools representing the 8 rows and 12 columns from this plate were grown up and their plasmid DNA extracted. These 20 preps were transfected separately into COS cells on 12 well plates (10^5 cells well, 4 wells per pool). 2-3 days after the transfection, labeled IL-12 was bound to the cells in two wells (total binding), whereas the other two wells per pool received labeled IL-12 and a 100 fold molar excess of cold IL-12 (= nonspecific binding). Wells were washed and the bound radioactivity eluted with 0.5 ml of 1% SDS and counted in a gamma counter. Two positive pools were identified in this manner, one representing column 1 and the other one representing row F from the microtiterplate. E. coli clones from well F1 must thus contain the IL-12 binding activity. Clones from that well were plated, and DNA from 10 single colonies was analyzed for plasmid insert size. 3 out of the 10 colonies showed an insert of about 2.1 kilobases in length, large enough to encode the IL-12 receptor. One of these clones was picked for further analysis.

Characterization of IL-12 receptor cDNAs

IL-12 receptor clone No. 5 was picked as described above and the plasmid DNA isolated. Gel isolated insert was sequenced on both strands using the ABI automated DNA sequencer in conjunction with a

thermostable DNA polymerase and dye-labeled dideoxynucleotides as terminators.

Sequence alignments were run using th ALIGN program (M. O. Dayhoff et al, Methods Enzymology 91,524,1983) with the mutation data matrix, a break penalty of 6 and 100 random runs.

Cloned IL-12 receptor cDNAs were expressed in COS cells using either the DEAE dextran transfection or electroporation techniques (Molecular Cloning, a Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbour Laboratory Press 1989). Binding assays with labeled IL-12 or labeled 2-4E6 antibody were run as described hereinabove under anti human IL-12 receptor antibody. The binding data were analyzed and Kd values were calculated according to Scatchard, using the Ligand program discussed hereinabove under anti human IL-12 receptor antibody. In vivo labeling (6 hours) of COS cells (3 x 10^5 cells per 35 mm diameter tissue culture dish) with ^{35}S Cysteine was performed 3 days after transfection as described (Molecular Cloning, a Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbour Laboratory Press 1989). Cells were washed in PBS and lysed in CHAPS lysis buffer (10 mM CHAPS - 300 mM NaCl - 50 mM Tris-HCl pH 7.4 - 2 mg/ml Iodoacetamide - 0.17 mg/ml PMSF), precleared by incubation with protein G Sepharose beads (50 ul packed beads per ml, Genex) and normal mouse serum (25 % final concentration) at 4 ^{0} C overnight. The beads were spun out and labeled IL-12 receptor was specifically immunoprecipitated from the cleared lysates by adding 5 ug of 2-4E6

antibody per ml of sample. The antibody was diluted in PBS containing 1% bovine serum albumin and had been loaded onto 50 ul of packed beads for 2 - 3 hours at 4 ^{0} C. Immunoprecipitation took place overnight at 4^{0} C. The next day, the beads were washed 3 - 4 times in CHAPS lysis buffer before analysis on SDS-polyacrylamide gels as described (Molecular Cloning, a Laboratory Manual, second edition, J. Sambrook, E.F. Fritsch, T. Maniatis, Cold Spring Harbour Laboratory Press 1989).

Sequence Analysis of IL-12 receptor cDNA clones

The DNA sequence for the IL-12 receptor cDNA insert from clone No. 5 is shown in Figure 1. The deduced amino acid sequence for the encoded receptor protein is shown in Figure 2. The IL-12 receptor protein is thus composed of 662 amino acids, and has the following features: N-terminal signal peptide, extracellular domain, transmembrane domain and cytoplasmic tail. The classical hydrophobic N-terminal signal peptide is predicted to be 20-24 amino acids in length. Signal peptide cleavage has been shown to occur mostly after the amino acids Ala, Ser, Gly, Cys, Thr, Gln (G. von Heijne, Nucl. Acids Research, 1986,14:4683) for the IL-12 receptor, the cleavage could thus take place after Gln20, Ala23 or Cys24 in the sequence shown in Figure 2. The extracellular domain of the receptor is predicted to encompass the region from the C-terminus of the signal peptide to amino acid No. 540 in the sequence shown in Figure 2. Hydrophobicity analysis shows the area from amino acid No. 541 to 571 to be

hydrophobic, as would be expected for a transmembrane anchor region. Charged transfer stop residues can be found at the N- as well as the C-terminus of this predicted TM area. This predicted extracellular domain also c ntains all 6 N-linked glycosylation sites. The cytoplasmic region is thus predicted to encompass amino acid residues No. 572 to 662.

Further analysis of the amino acid sequence of the IL-12 receptor shows it to be a member of the cytokine receptor superfamily, by virtue of the sequence motifs [Cys52 --- Cys62SW] and [W222SKWS]. Comparing the IL-12 receptor sequence to all the members of the superfamily by running the ALIGN program shows that the IL-12 receptor has the highest homology to human GP130.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Chua, Anne O
Gubler, Ulrich A

(ii) TITLE OF INVENTION: INTERLEUKIN-12 RECEPTOR

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Hoffmann-La Roche Inc.
(B) STREET: 340 Kingsland Street
(C) CITY: Nutley
(D) STATE: New Jersey
(E) COUNTRY: United States of America
(F) ZIP: 07110-1199

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Kass, Alan P
(B) REGISTRATION NUMBER: 32142
(C) REFERENCE/DOCKET NUMBER: CD 8828

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (201) 235-4205
(B) TELEFAX: (201) 235-3500

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2104 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens
(G) CELL TYPE: human T-cells

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: library 3 day PHA/pEF-BOS
(B) CLONE: human interleukin-12 receptor clone #5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 65..2050

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GGTGGCTGAA CCTCGCAGGT GGCAGAGAGG CTCCCCTGGG GCTGTGGGGC TCTACGTGGA      60

TCCG ATG GAG CCG CTC GTG ACC TGG GTG GTC CCC CTC CTC TTC CTC TTC      109
     Met Glu Pro Pro Val Thr Trp Val Val Pro Leu Leu Phe Leu Phe
       1               5                  10                  15

CTG CTG TCC AGG CAG GGC GCT GCC TGC AGA ACC AGT GAG TGC TGT TTT      157
Leu Leu Ser Arg Gln Gly Ala Ala Cys Arg Thr Ser Glu Cys Cys Phe
                   20                  25                  30

CAG GAC CCG CCA TAT CCG GAT GCA GAC TCA GGC TCG GCC TCG GGC CCT      205
Gln Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro
               35                  40                  45

AGG GAC CTG AGA TGC TAT CGG ATA TCC AGT GAT CGT TAC GAG TGC TCC      253
Arg Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser
           50                  55                  60

TGG CAG TAT GAG GGT CCC ACA GCT GGG GTC AGC CAC TTC CTG CGG TGT      301
Trp Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys
       65                  70                  75

TGC CTT AGC TCC GGG CGC TGC TGC TAC TTC GCC GCC GGC TCA GCC ACC      349
Cys Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr
 80                  85                  90                  95

AGG CTG CAG TTC TCC GAC CAG GCT GGG GTG TCT GTG CTG TAC ACT GTC      397
Arg Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val
                100                 105                 110

ACA CTC TGG GTG GAA TCC TGG GCC AGG AAC CAG ACA GAG AAG TCT CCT      445
Thr Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro
            115                 120                 125

GAG GTG ACC CTG CAG CTC TAC AAC TCA GTT AAA TAT GAG CCT CCT CTG      493
Glu Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu
        130                 135                 140

GGA GAC ATC AAG GTG TCC AAG TTG GCC GGG CAG CTG CGT ATG GAG TGG      541
Gly Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp
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                145                 150                 155

GAG ACC CCG GAT AAC CAG GTT GGT GCT GAG GTG CAG TTC CGG CAC CGG      589
Glu Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg
160                 165                 170                 175

ACA CCC AGC AGC CCA TGG AAG TTG GGC GAC TGC GGA CCT CAG GAT GAT      637
Thr Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp
                180                 185                 190

GAT ACT GAG TCC TGC CTC TGC CCC CTG GAG ATG AAT GTG GCC CAG GAA      685
Asp Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu
            195                 200                 205

TTC CAG CTC CGA CGA CGG CAG CTG GGG AGC CAA GGA AGT TCC TGG AGC      733
Phe Gln Leu Arg Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser
        210                 215                 220

AAG TGG AGC AGC CCC GTG TGC GTT CCC CCT GAA AAC CCC CCA CAG CCT      781
Lys Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro
    225                 230                 235

CAG GTG AGA TTC TCG GTG GAG CAG CTG GGC CAG GAT GGG AGG AGG CGG      829
Gln Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg
240                 245                 250                 255

CTG ACC CTG AAA GAG CAG CCA ACC CAG CTG GAG CTT CCA GAA GGC TGT      877
Leu Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys
                260                 265                 270

CAA GGG CTG GCG CCT GGC ACG GAG GTC ACT TAC CGA CTA CAG CTC CAC      925
Gln Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His
            275                 280                 285

ATG CTG TCC TGC CCG TGT AAG GCC AAG GCC ACC AGG ACC CTG CAC CTG      973
Met Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu
        290                 295                 300

GGG AAG ATG CCC TAT CTC TCG GGT GCT GCC TAC AAC GTG GCT GTC ATC     1021
Gly Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile
    305                 310                 315

TCC TCG AAC CAA TTT GGT CCT GGC CTG AAC CAG ACG TGG CAC ATT CCT     1069
Ser Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro
320                 325                 330                 335

GCC GAC ACC CAC ACA GAA CCA GTG GCT CTG AAT ATC AGC GTC GGA ACC     1117
Ala Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr
                340                 345                 350

AAC GGC ACC ACC ATG TAT TGG CCA GCC CGG GCT CAG AGC ATG ACG TAT     1165
Asn Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr
            355                 360                 365
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TGC ATT GAA TGG CAG CCT GTG GGC CAG GAC GGG GGC CTT GCC ACC TGC      1213
Cys Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys
        370                 375                 380

AGC CTG ACT GCG CCG CAA GAC CCG GAT CCG GCT GGA ATG GCA ACC TAC      1261
Ser Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr
    385                 390                 395

AGC TGG AGT CGA GAG TCT GGG GCA ATG GGG CAG GAA AAG TGT TAC TAC      1309
Ser Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr
400                 405                 410                 415

ATT ACC ATC TTT GCC TCT GCG CAC CCC GAG AAG CTC ACC TTG TGG TCT      1357
Ile Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser
                420                 425                 430

ACG GTC CTG TCC ACC TAC CAC TTT GGG GGC AAT GCC TCA GCA GCT GGG      1405
Thr Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly
            435                 440                 445

ACA CCG CAC CAC GTC TCG GTG AAG AAT CAT AGC TTG GAC TCT GTG TCT      1453
Thr Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser
        450                 455                 460

GTG GAC TGG GCA CCA TCC CTG CTG AGC ACC TGT CCC GGC GTC CTA AAG      1501
Val Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys
    465                 470                 475

GAG TAT GTT GTC CGC TGC CGA GAT GAA GAC AGC AAA CAG GTG TCA GAG      1549
Glu Tyr Val Val Arg Cys Arg Asp Glu Asp Ser Lys Gln Val Ser Glu
480                 485                 490                 495

CAT CCC GTG CAG CCC ACA GAG ACC CAA GTT ACC CTC AGT GGC CTG CGG      1597
His Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg
                500                 505                 510

GCT GGT GTA GCC TAC ACG GTG CAG GTG CGA GCA GAC ACA GCG TGG CTG      1645
Ala Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu
            515                 520                 525

AGG GGT GTC TGG AGC CAG CCC CAG CGC TTC AGC ATC GAA GTG CAG GTT      1693
Arg Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val
        530                 535                 540

TCT GAT TGG CTC ATC TTC TTC GCC TCC CTG GGG AGC TTC CTG AGC ATC      1741
Ser Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile
    545                 550                 555

CTT CTC GTG GGC GTC CTT GGC TAC CTT GGC CTG AAC AGG GCC GCA CGG      1789
Leu Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg
560                 565                 570                 575

CAC CTG TGC CCG CCG CTG CCC ACA CCC TGT GCC AGC TCC GCC ATT GAG      1837
His Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu
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                580                 585                 590

TTC CCT GGA GGG AAG GAG ACT TGG CAG TGG ATC AAC CCA GTG GAC TTC      1885
Phe Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe
            595                 600                 605

CAG GAA GAG GCA TCC CTG CAG GAG GCC CTG GTG GTA GAG ATG TCC TGG      1933
Gln Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp
        610                 615                 620

GAC AAA GGC GAG AGG ACT GAG CCT CTC GAG AAG ACA GAG CTA CCT GAG      1981
Asp Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu
    625                 630                 635

GGT GCC CCT GAG CTG GCC CTG GAT ACA GAG TTG TCC TTG GAG GAT GGA      2029
Gly Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly
640                 645                 650                 655

GAC AGG TGC AAG GCC AAG ATG TGATCGTTGA GGCTCAGAGA GGGTGAGTGA         2080
Asp Arg Cys Lys Ala Lys Met
                660

CTCGCCCGAG GCTACGTAGC CTTT                                          2104
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 662 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 1..23
(D) OTHER INFORMATION: /note= "N-terminal signal peptide (1..20 or 23 or 24)"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 541..570
(D) OTHER INFORMATION: /note= "transmembrane region"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 571..662
(D) OTHER INFORMATION: /note= "cytoplasmic tail region"

(ix) FEATURE:
(A) NAME/KEY: Region

(B) LOCATION: 52..64
(D) OTHER INFORMATION: /note= "sequence motif of cytokine receptor superfamily Cys52..Cys62SW"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 222..226
(D) THER INFORMATION: /note= "cytokine receptor superfamily motif (W222SKWS)"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 121..123
(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 329..331
(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 346..348
(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 352..354
(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 442..444
(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 456..458
(D) OTHER INFORMATION: /note= "N-linked glycosylation site"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 24..540
(D) OTHER INFORMATION: /note= "Extracellular region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Pro Leu Val Thr Trp Val Val Pro Leu Leu Phe Leu Phe Leu
1               5                  10                  15

Leu Ser Arg Gln Gly Ala Ala Cys Arg Thr Ser Glu Cys Cys Phe Gln
            20                  25                  30

Asp Pro Pro Tyr Pro Asp Ala Asp Ser Gly Ser Ala Ser Gly Pro Arg
        35                  40                  45

Asp Leu Arg Cys Tyr Arg Ile Ser Ser Asp Arg Tyr Glu Cys Ser Trp
    50                  55                  60

Gln Tyr Glu Gly Pro Thr Ala Gly Val Ser His Phe Leu Arg Cys Cys
65                  70                  75                  80

Leu Ser Ser Gly Arg Cys Cys Tyr Phe Ala Ala Gly Ser Ala Thr Arg
                85                  90                  95

Leu Gln Phe Ser Asp Gln Ala Gly Val Ser Val Leu Tyr Thr Val Thr
            100                 105                 110

Leu Trp Val Glu Ser Trp Ala Arg Asn Gln Thr Glu Lys Ser Pro Glu
        115                 120                 125

Val Thr Leu Gln Leu Tyr Asn Ser Val Lys Tyr Glu Pro Pro Leu Gly
    130                 135                 140

Asp Ile Lys Val Ser Lys Leu Ala Gly Gln Leu Arg Met Glu Trp Glu
145                 150                 155                 160

Thr Pro Asp Asn Gln Val Gly Ala Glu Val Gln Phe Arg His Arg Thr
                165                 170                 175

Pro Ser Ser Pro Trp Lys Leu Gly Asp Cys Gly Pro Gln Asp Asp Asp
            180                 185                 190

Thr Glu Ser Cys Leu Cys Pro Leu Glu Met Asn Val Ala Gln Glu Phe
        195                 200                 205

Gln Leu Arg Arg Arg Gln Leu Gly Ser Gln Gly Ser Ser Trp Ser Lys
    210                 215                 220

Trp Ser Ser Pro Val Cys Val Pro Pro Glu Asn Pro Pro Gln Pro Gln
225                 230                 235                 240

Val Arg Phe Ser Val Glu Gln Leu Gly Gln Asp Gly Arg Arg Arg Leu
                245                 250                 255

Thr Leu Lys Glu Gln Pro Thr Gln Leu Glu Leu Pro Glu Gly Cys Gln
            260                 265                 270

Gly Leu Ala Pro Gly Thr Glu Val Thr Tyr Arg Leu Gln Leu His Met
        275                 280                 285
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Leu Ser Cys Pro Cys Lys Ala Lys Ala Thr Arg Thr Leu His Leu Gly
    290                 295                 300

Lys Met Pro Tyr Leu Ser Gly Ala Ala Tyr Asn Val Ala Val Ile Ser
305                 310                 315                 320

Ser Asn Gln Phe Gly Pro Gly Leu Asn Gln Thr Trp His Ile Pro Ala
                325                 330                 335

Asp Thr His Thr Glu Pro Val Ala Leu Asn Ile Ser Val Gly Thr Asn
            340                 345                 350

Gly Thr Thr Met Tyr Trp Pro Ala Arg Ala Gln Ser Met Thr Tyr Cys
        355                 360                 365

Ile Glu Trp Gln Pro Val Gly Gln Asp Gly Gly Leu Ala Thr Cys Ser
    370                 375                 380

Leu Thr Ala Pro Gln Asp Pro Asp Pro Ala Gly Met Ala Thr Tyr Ser
385                 390                 395                 400

Trp Ser Arg Glu Ser Gly Ala Met Gly Gln Glu Lys Cys Tyr Tyr Ile
                405                 410                 415

Thr Ile Phe Ala Ser Ala His Pro Glu Lys Leu Thr Leu Trp Ser Thr
            420                 425                 430

Val Leu Ser Thr Tyr His Phe Gly Gly Asn Ala Ser Ala Ala Gly Thr
        435                 440                 445

Pro His His Val Ser Val Lys Asn His Ser Leu Asp Ser Val Ser Val
    450                 455                 460

Asp Trp Ala Pro Ser Leu Leu Ser Thr Cys Pro Gly Val Leu Lys Glu
465                 470                 475                 480

Tyr Val Val Arg Cys Arg Asp Glu Asp Ser Lys Gln Val Ser Glu His
                485                 490                 495

Pro Val Gln Pro Thr Glu Thr Gln Val Thr Leu Ser Gly Leu Arg Ala
            500                 505                 510

Gly Val Ala Tyr Thr Val Gln Val Arg Ala Asp Thr Ala Trp Leu Arg
        515                 520                 525

Gly Val Trp Ser Gln Pro Gln Arg Phe Ser Ile Glu Val Gln Val Ser
    530                 535                 540

Asp Trp Leu Ile Phe Phe Ala Ser Leu Gly Ser Phe Leu Ser Ile Leu
545                 550                 555                 560

Leu Val Gly Val Leu Gly Tyr Leu Gly Leu Asn Arg Ala Ala Arg His
                565                 570                 575
```

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Leu Cys Pro Pro Leu Pro Thr Pro Cys Ala Ser Ser Ala Ile Glu Phe
            580                 585                 590

Pro Gly Gly Lys Glu Thr Trp Gln Trp Ile Asn Pro Val Asp Phe Gln
        595                 600                 605

Glu Glu Ala Ser Leu Gln Glu Ala Leu Val Val Glu Met Ser Trp Asp
    610                 615                 620

Lys Gly Glu Arg Thr Glu Pro Leu Glu Lys Thr Glu Leu Pro Glu Gly
625                 630                 635                 640

Ala Pro Glu Leu Ala Leu Asp Thr Glu Leu Ser Leu Glu Asp Gly Asp
                645                 650                 655

Arg Cys Lys Ala Lys Met
            660
```

CLAIMS:

1. A substantially pure, homogeneous and isolated DNA sequence encoding for a lower affinity Interleukin-12 receptor, or a fragment of said low affinity Interleukin-12 receptor which binds specifically to Interleukin-12.

2. The DNA sequence of claim 1 encoding for the human low affinity Interleukin-12 receptor, or a fragment of said human low affinity Interleukin-12 receptor which binds specifically to Interleukin-12.

3. The DNA sequence of claim 2, SEQ ID NO:1 or a degenerate variant thereof.

4. The DNA sequence of claim 3 which encodes an Interleukin-12 receptor protein exhibiting Interleukin-12 binding activity, said protein having the amino acid sequence SEQ ID NO:2 or a portion thereof which exhibits Interleukin-12 binding activity.

5. A recombinant expression vector comprising a substantially pure, homogeneous and isolated DNA sequence encoding for a low affinity Interleukin-12 receptor, or a fragment of said low affinity Interleukin-12 receptor which binds specifically to Interleukin-12.

6. The recombinant expression vector of claim 5 comprising a DNA sequence encoding for the human l w affinity Interleukin-12 receptor. or a fragm nt of said human low affinity Interleukin-12 receptor which binds specifically to Interleukin-12.

7. The recombinant expression vector of claim 6 wherein the DNA sequence is SEQ ID NO:1 or a degenerate variant thereof.

8. The recombinant expression vector of claim 7 wherein the DNA sequence encodes an Interleukin-12 receptor protein exhibiting Interleukin-12 binding activity, said protein having the amino acid sequence SEQ ID NO:2 or a portion thereof which exhibits Interleukin-12 binding activity.

9. A host cell which has been transformed by a recombinant expression vector comprising a substantially pure, homogeneous and isolated DNA sequence encoding for a low affinity Interleukin-12 receptor, or a fragment of said low affinity Interleukin-12 receptor which binds specifically to Interleukin-12.

10. The host cell of claim 9 wherein the recombinant expression vector comprises a DNA sequence encoding for the human low affinity Interleukin-12 receptor, or a fragment of said human low affinity Interleukin-12 receptor which binds specfically to Interleukin-12.

11. The host cell of claim 10 wherein the DNA sequence is SEQ ID NO:1 or a degenerate variant thereof.

12. The host cell of claim 11 wherein the DNA sequence encodes an Interleukin-12 receptor protein exhibiting Interleukin-12 binding activity, said protein having th amino acid sequence SEQ ID NO:2 or a portion thereof which encodes a soluble, truncated Interleukin-12 receptor protein which exhibits Interleukin-12 binding activity.

13. The host cell of claim 9 which is a mammalian cell.

14. A substantially pure, homogeneous and isolated low affinity Interleukin-12 receptor protein, or a fragment of said low affinity Interleukin-12 receptor protein which binds specifically to Interleukin-12.

15. The Interleukin-12 receptor protein of claim 14 being the human low affinity Interleukin-12 receptor protein, or a fragment of said human low affinity Interleukin-12 receptor protein which binds specifically to Interleukin-12.

16. The Interleukin-12 receptor protein of claim 15, SEQ ID NO:2 and mutants thereof.

17. The Interleukin-12 receptor protein of claim 16 having a molecular weight of about 150 kilodaltons.

18. A pharmaceutical composition comprising a substantially pure, homogeneous and isolated low affinity Interleukin-12 receptor pr tein, or a fragment of said low affinity Interleukin-12 receptor protein which binds specifically to Interleukin-12 and a suitable diluent or carrier.

19. The pharmaceutical composition of claim 18 wherein the low affinity Interleukin-12 receptor protein is the human low affinity Interleukin-12 receptor protein, or a fragment of said human low affinity Interleukin-12 receptor protein which binds specifically to Interleukin-12.

20. The pharmaceutical composition of claim 19 wherein the human low affinity Interleukin-12 receptor protein is SEQ ID NO:2.

ABSTRACT

This invention relates to substantially pure Interleukin-12 receptor cDNAs and protein and uses therefore. The Interleukin-12 receptor is shown to be a member of the cytokine receptor superfamily and has a high homology to human gp130.

Attorney Docket No. 8828

Declaration and Power of Attorney for Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

HUMAN RECEPTOR FOR INTERLEUKIN-12

the specification of which

(check one)

[X] is attached hereto.

[ ] was filed on _____ as

Application Serial No. _____

and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
(Number)	(Country)	(Day/Month/Year Filed)	[ ] Yes	[ ] No
(Number)	(Country)	(Day/Month/Year Filed)	[ ] Yes	[ ] No
(Number)	(Country)	(Day/Month/Year Filed)	[ ] Yes	[ ] No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the clams of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

George M. Gould	(Reg. No. 20970)	William H. Epstein	(Reg. No. 20008)
Bernard S. Leon	(Reg. No: ~~20756~~)	William G. Isgro	(Reg. No. 22041)
Alan P. Kass	(Reg. No. 32142)	George W. Johnston	(Reg. No. 28090)

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Anne On Chua

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Third Inventor's signature — Date

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Citizenship

Post Office Address

Full name of fourth joint inventor, if any

Fourth Inventor's signature — Date

Residence

Citizenship

Post Office Address

Full name of fifth joint inventor, if any

Fifth Inventor's signature Date

Residence

Citizenship

Post Office Address

Title 37, Code of Federal Regulations, §1.56
Duty to disclose information material to patentability.

(a) Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie of unpatentability of a claim: or

(2) It refuses, or is inconsistent with, a position the applicant takes in:
(i) Opposing an argument of unpatentability relied on by the Office, or
(ii) Asserting an argument of patentability.

Figure 1

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```
        10         20         30         40         50         60         70
GGTGGCTGAA CCTCGCAGGT GGCAGAGAGG CTCCCCTGGG GCTGTGGGGC TCTACGTGGA TCCGATGGAG

        80         90        100        110        120        130        140
CCGCTGGTGA CCTGGGTGGT CCCCCTCCTC TTCCTCTTCC TGCTGTCCAG GCAGGGCGCT GCCTGCAGAA

       150        160        170        180        190        200        210
CCAGTGAGTG CTGTTTTCAG GACCCGCCAT ATCCGGATGC AGACTCAGGC TCGGCCTCGG GCCCTAGGGA

       220        230        240        250        260        270        280
CCTGAGATGC TATCGGATAT CCAGTGATCG TTACGAGTGC TCCTGGCAGT ATGAGGGTCC CACAGCTGGG

       290        300        310        320        330        340        350
GTCAGCCACT TCCTGCGGTG TTGCCTTAGC TCCGGGCGCT GCTGCTACTT CGCCGCCGGC TCAGCCACCA

       360        370        380        390        400        410        420
GGCTGCAGTT CTCCGACCAG GCTGGGGTGT CTGTGCTGTA CACTGTCACA CTCTGGGTGG AATCCTGGGC

       430        440        450        460        470        480        490
CAGGAACCAG ACAGAGAAGT CTCCTGAGGT GACCCTGCAG CTCTACAACT CAGTTAAATA TGAGCCTCCT

       500        510        520        530        540        550        560
CTGGGAGACA TCAAGGTGTC CAAGTTGGCC GGGCAGCTGC GTATGGAGTG GGAGACCCCG GATAACCAGG

       570        580        590        600        610        620        630
TTGGTGCTGA GGTGCAGTTC CGGCACCGGA CACCCAGCAG CCCATGGAAG TTGGGCGACT GCGGACCTCA

       640        650        660        670        680        690        700
GGATGATGAT ACTGAGTCCT GCCTCTGCCC CCTGGAGATG AATGTGGCCC AGGAATTCCA GCTCCGACGA

       710        720        730        740        750        760        770
CGGCAGCTGG GGAGCCAAGG AAGTTCCTGG AGCAAGTGGA GCAGCCCCGT GTGCGTTCCC CCTGAAAACC

       780        790        800        810        820        830        840
CCCCACAGCC TCAGGTGAGA TTCTCGGTGG AGCAGCTGGG CCAGGATGGG AGGAGGCGGC TGACCCTGAA

       850        860        870        880        890        900        910
AGAGCAGCCA ACCCAGCTGG AGCTTCCAGA AGGCTGTCAA GGGCTGGCGC CTGGCACGGA GGTCACTTAC

       920        930        940        950        960        970        980
CGACTACAGC TCCACATGCT GTCCTGCCCG TGTAAGGCCA AGGCCACCAG GACCCTGCAC CTGGGGAAGA

       990       1000       1010       1020       1030       1040       1050
TGCCCTATCT CTCGGGTGCT GCCTACAACG TGGCTGTCAT CTCCTCGAAC CAATTTGGTC CTGGCCTGAA

      1060       1070       1080       1090       1100       1110       1120
CCAGACGTGG CACATTCCTG CCGACACCCA CACAGAACCA GTGGCTCTGA ATATCAGCGT CGGAACCAAC

      1130       1140       1150       1160       1170       1180       1190
GGGACCACCA TGTATTGGCC AGCCCGGGCT CAGAGCATGA CGTATTGCAT TGAATGGCAG CCTGTGGGCC

      1200       1210       1220       1230       1240       1250       1260
AGGACGGGGG CCTTGCCACC TGCAGCCTGA CTGCGCCGCA AGACCCGGAT CCGGCTGGAA TGGCAACCTA

      1270       1280       1290       1300       1310       1320       1330
CAGCTGGAGT CGAGAGTCTG GGGCAATGGG GCAGGAAAAG TGTTACTACA TTACCATCTT TGCCTCTGCG
```

```
      1340        1350        1360        1370        1380        1390        1400
CAC--CGAGA AGCTCACCTT GTGGTCTACG GTCCTGTCCA CCTACCACTT TGGGGGCAAT GCCTCAGCAG

      1410        1420        1430        1440        1450        1460        1470
CTGGGACACC GCACCACGTC TCGGTGAAGA ATCATAGCTT GGACTCTGTG TCTGTGGACT GGGCACCATC

      1480        1490        1500        1510        1520        1530        1540
CCTGCTGAGC ACCTGTCCCG GCGTCCTAAA GGAGTATGTT GTCCGCTGCC GAGATGAAGA CAGCAAACAG

      1550        1560        1570        1580        1590        1600        1610
GTGTCAGAGC ATCCCGTGCA GCCCACAGAG ACCCAAGTTA CCCTCAGTGG CCTGCGGGCT GGTGTAGCCT

      1620        1630        1640        1650        1660        1670        1680
ACACGGTGCA GGTGCGAGCA GACACAGCGT GGCTGAGGGG TGTCTGGAGC CAGCCCCAGC GCTTCAGCAT

      1690        1700        1710        1720        1730        1740        1750
CGAAGTGCAG GTTTCTGATT GGCTCATCTT CTTCGCCTCC CTGGGGAGCT TCCTGAGCAT CCTTCTCGTG

      1760        1770        1780        1790        1800        1810        1820
GGCGTCCTTG GCTACCTTGG CCTGAACAGG GCCGCACGGC ACCTGTGCCC GCCGCTGCCC ACACCCTGTG

      1830        1840        1850        1860        1870        1880        1890
CCAGCTCCGC CATTGAGTTC CCTGGAGGGA AGGAGACTTG GCAGTGGATC AACCCAGTGG ACTTCCAGGA

      1900        1910        1920        1930        1940        1950        1960
AGAGGCATCC CTGCAGGAGG CCCTGGTGGT AGAGATGTCC TGGGACAAAG GCGAGAGGAC TGAGCCTCTC

      1970        1980        1990        2000        2010        2020        2030
GAGAAGACAG AGCTACCTGA GGGTGCCCCT GAGCTGGCCC TGGATACAGA GTTGTCCTTG GAGGATGGAG

      2040        2050        2060        2070        2080        2090        2100
ACAGGTGCAA GGCCAAGATG TGATCGTTGA GGCTCAGAGA GGGTGAGTGA CTCGCCCGAG GCTACGTAGC
```

FIGURE 2

```
        10         20         30         40         50         60         70
MEPLVTWVVP LLFLFLLSRQ GAACRTSECC FQDPPYPDAD SGSASGPRDL RCYRISSDRY ECSWQYEGPT

        80         90        100        110        120        130        140
AGVSHFLRCC LSSGRCCYFA AGSATRLQFS DQAGVSVLYT VTLWVESWAR NQTEKSPEVT LQLYNSVKYE

       150        160        170        180        190        200        210
PPLGDIKVSK LAQQLRMEWE TPDNQVGAEV QFRHRTPSSP WKLGDCGPQD DDTESCLCPL EMNVAQEFQL

       220        230        240        250        260        270        280
RRRQLGSQGS SWSKWSSPVC VPPENPPQPQ VRFSVEQLGQ DGRRRLTLKE QPTQLELPEG CQGLAPGTEV

       290        300        310        320        330        340        350
TYRLQLHMLS CPCKAKATRT LHLGKMPYLS GAAYNVAVIS SNQFGPGLNQ TWHIPADTHT EPVALNISVG

       360        370        380        390        400        410        420
TNGTTMYWPA RAQSMTYCIE WQPVGQDGGL ATCSLTAPQD PDPAGMATYS WSRESGAMGQ EKCYYITIFA

       430        440        450        460        470        480        490
SAHPEKLTLW STVLSTYHFG GNASAAGTPH HVSVKNHSLD SVSVDWAPSL LSTCPGVLKE YVVRCRDEDS

       500        510        520        530        540        550        560
KQVSEHPVQP TETQVTLSGL RAGVAYTVQV RADTAWLRGV WSQPQRFSIE VQVSDWLIFF ASLGSFLSIL

       570        580        590        600        610        620        630
LVGVLGYLGL NRAARHLCPP LPTPCASSAI EFPGGKETWQ WINPVDFQEE ASLQEALVVE MSWDKGERTE

       640        650        660
PLEKTELPEG APELALDTEL SLEDGDRCKA KM
```

Figure 1A:

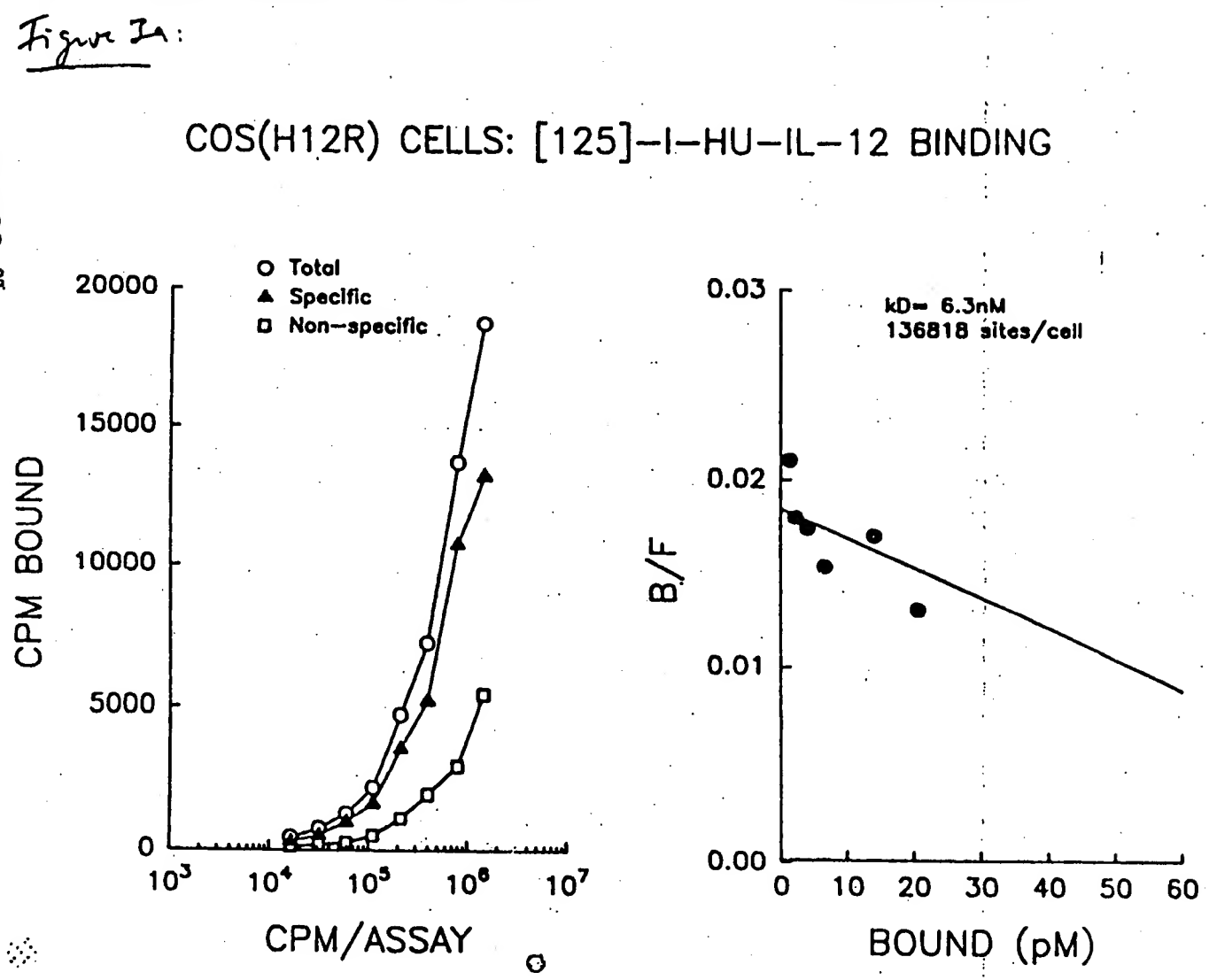


Figure 3b:

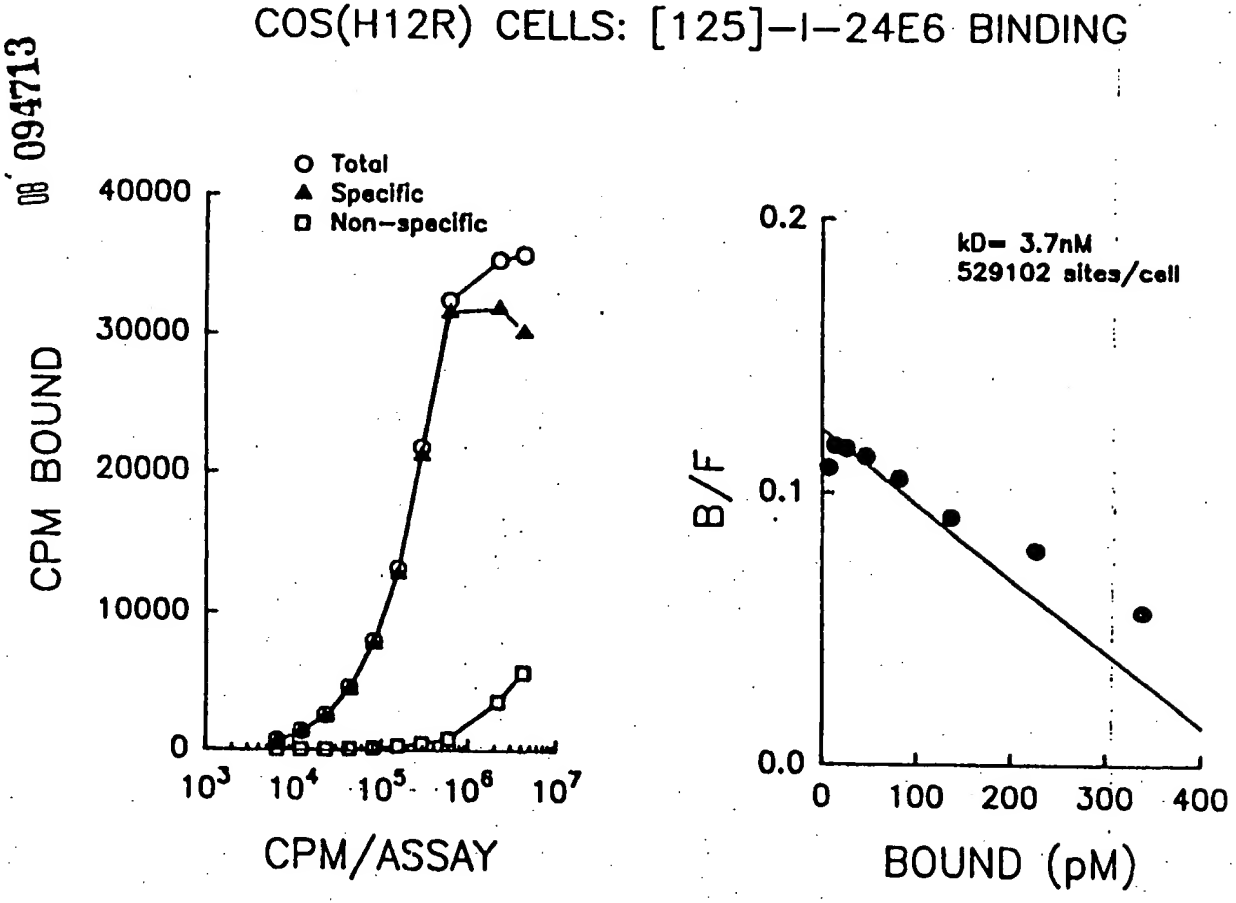

COS(H12R) CELLS: [125]-I-24E6 BINDING
08 094713
Total
Specific
Non-specific
CPM BOUND
40000
30000
20000
10000
0
10^3
10^4
10^5
10^6
10^7
CPM/ASSAY
kD= 3.7nM
529102 sites/cell
B/F
0.2
0.1
0.0
0
100
200
300
400
BOUND (pM)

Figure 4

08 094713

figure 2
MARKERS
7.4
5.3
2.8
1.9
1.6
PBMC - PHA
PBMC + PHA
KIT 225/K6
PBMC + PHA
origin
094713

INHIBITION OF [125]–I–IL–12 RECEPTOR BINDING BY MOUSE ANTI IL–12 RECEPTOR ANTISERUM

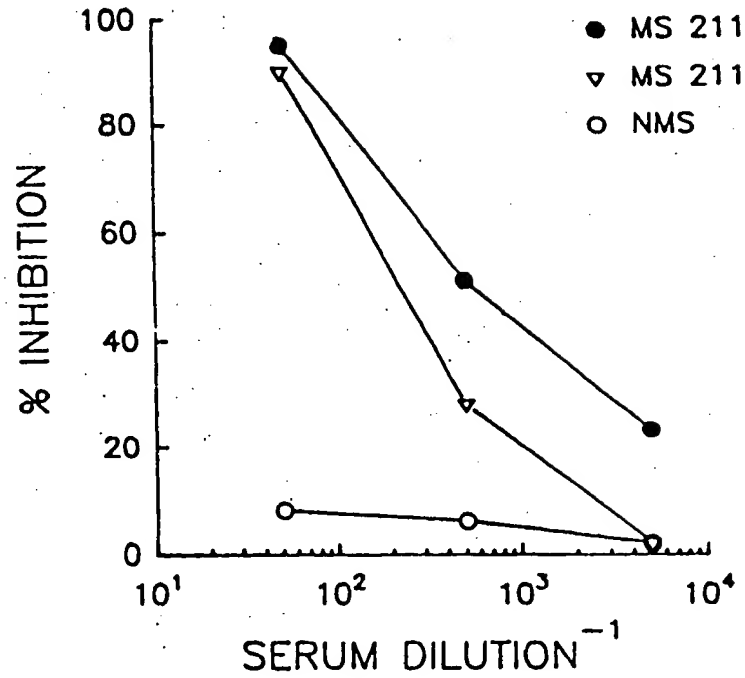
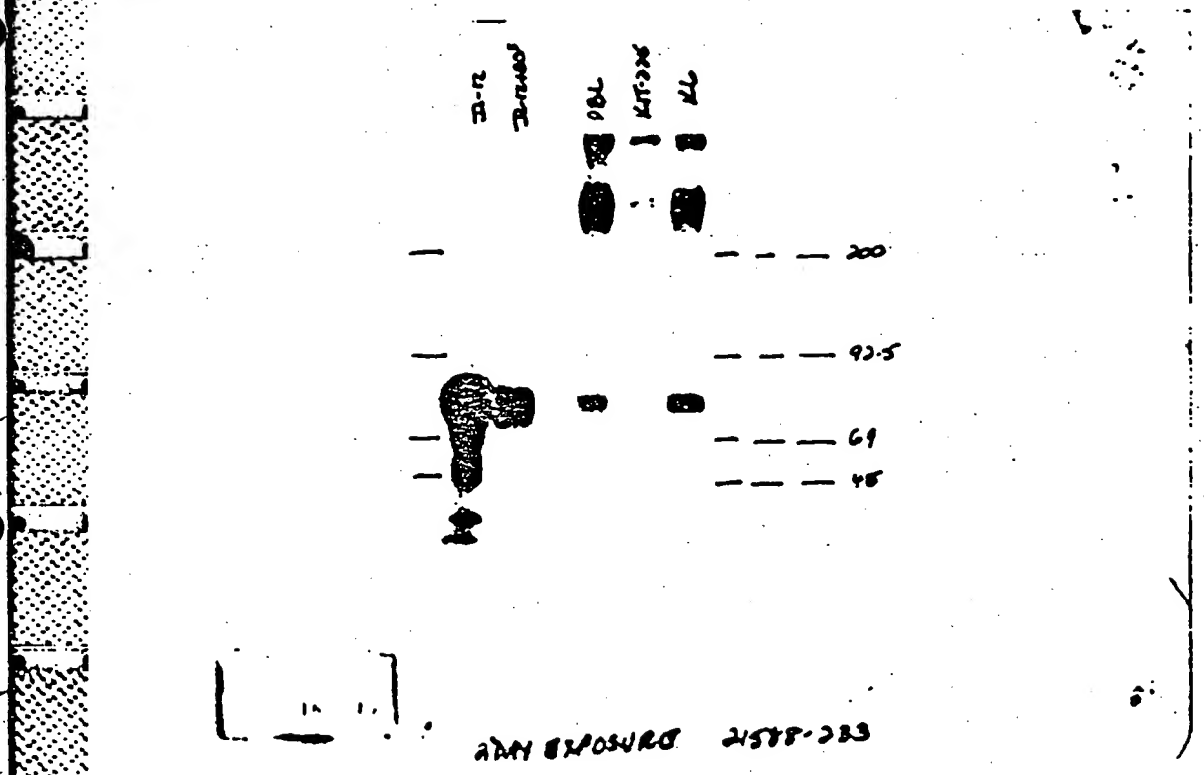
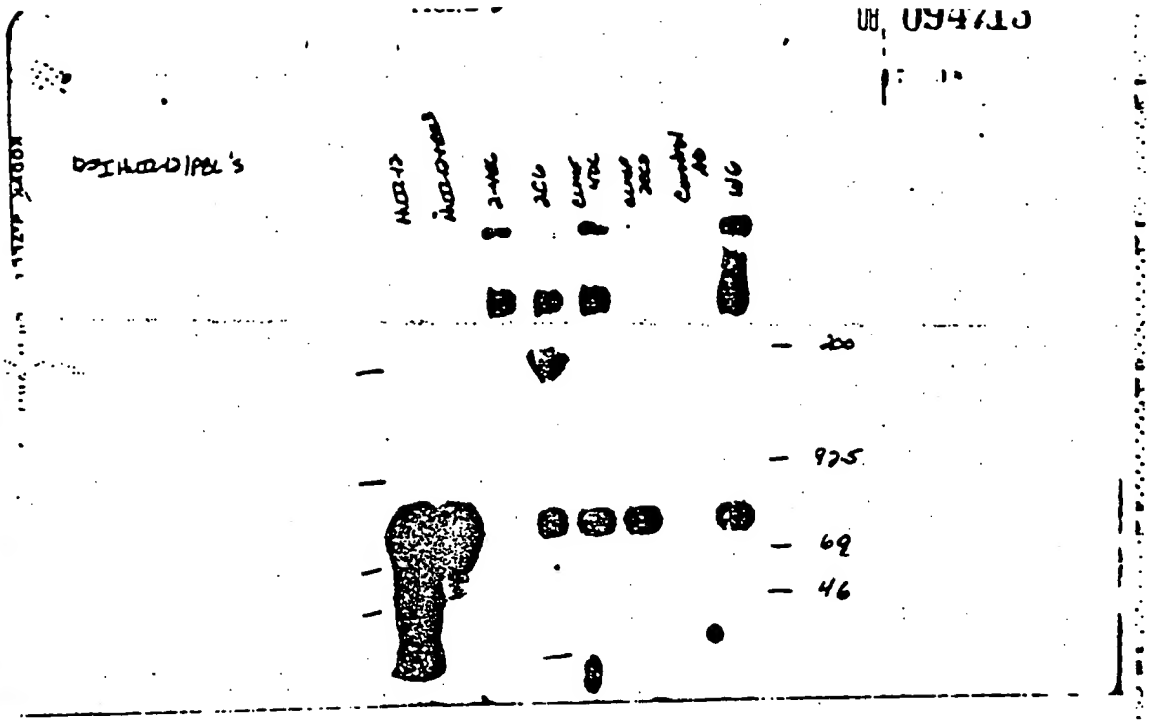


FIGURE 6

FIGURE 7




DEHALOGENASE's
200
92.5
69
46

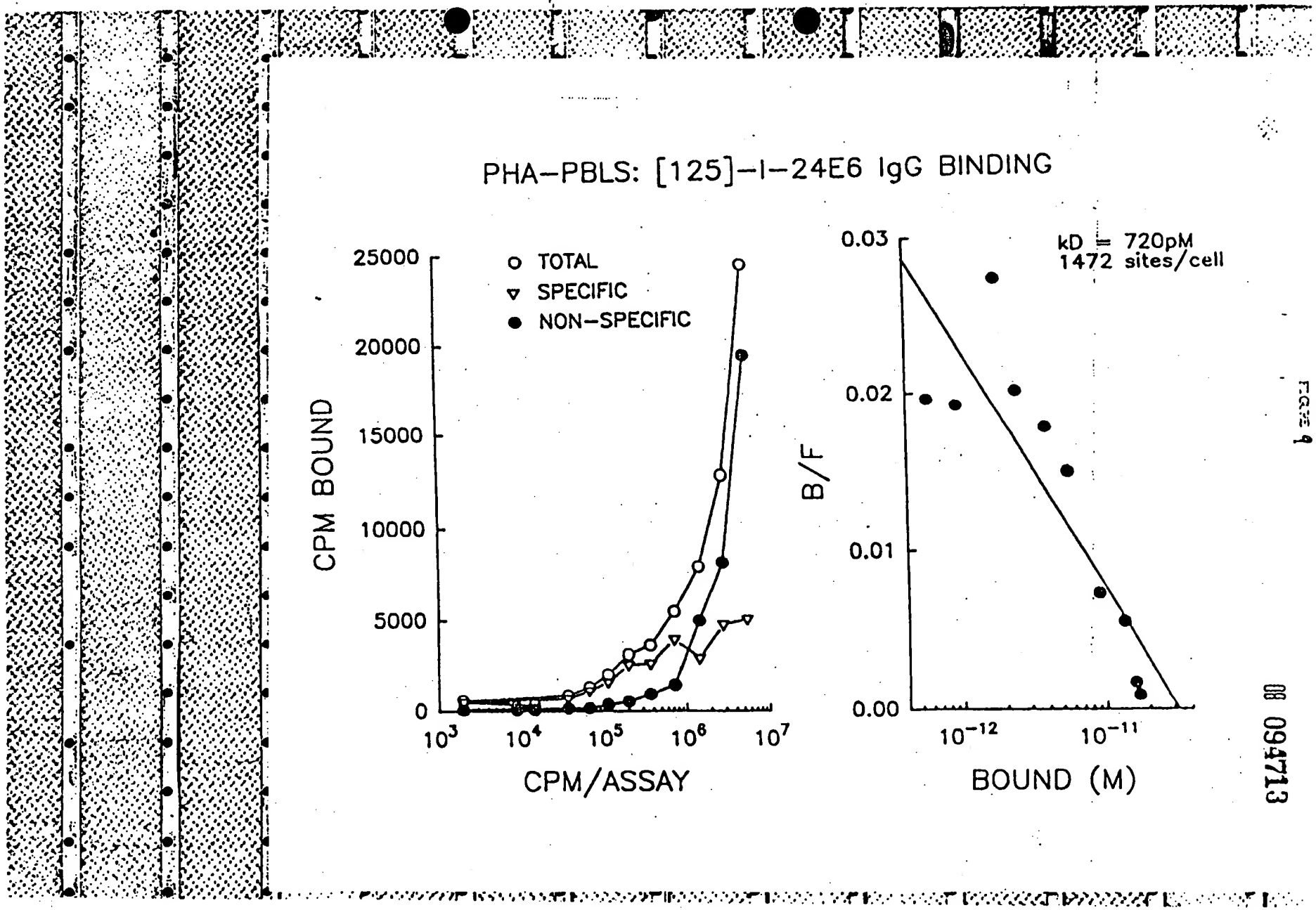


FIGURE 9

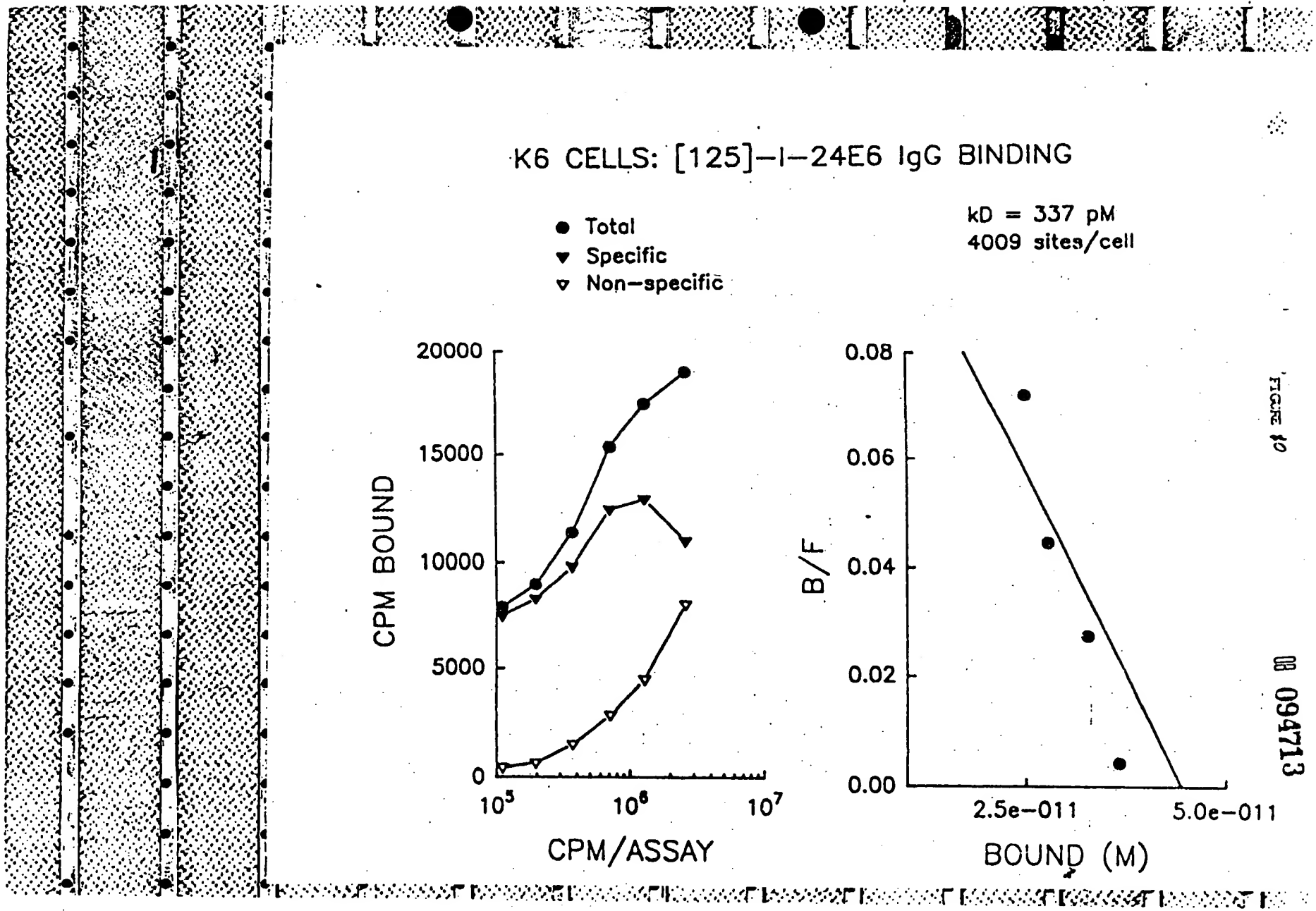


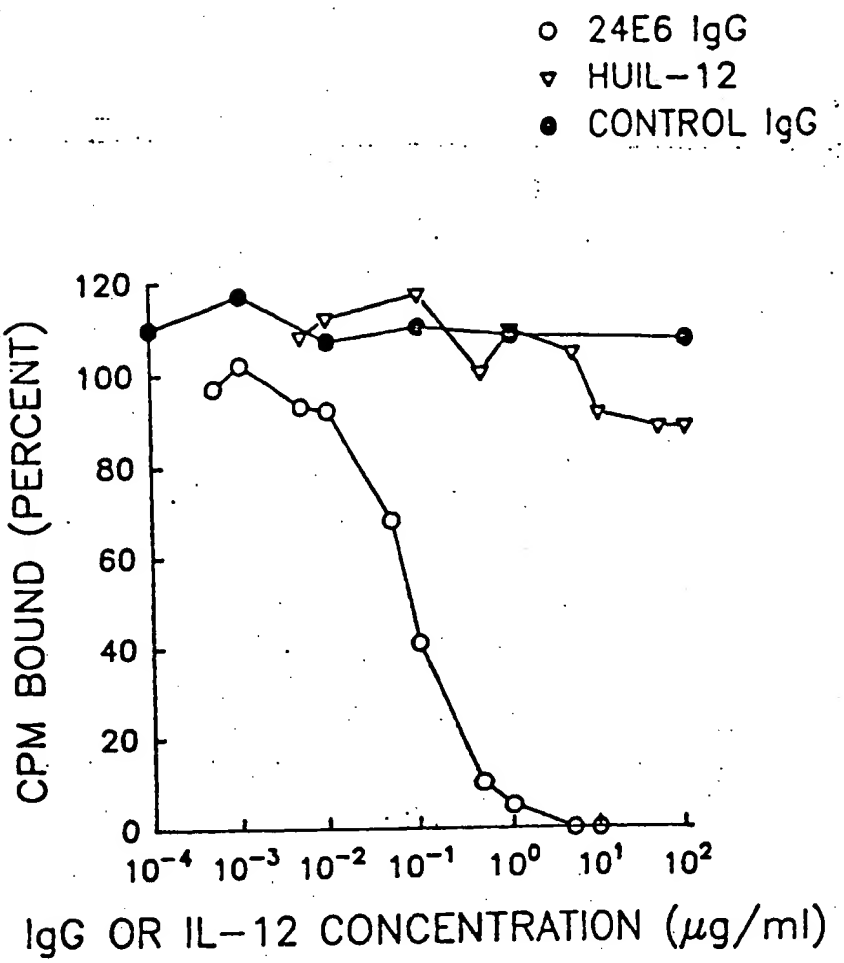
FIGURE 10

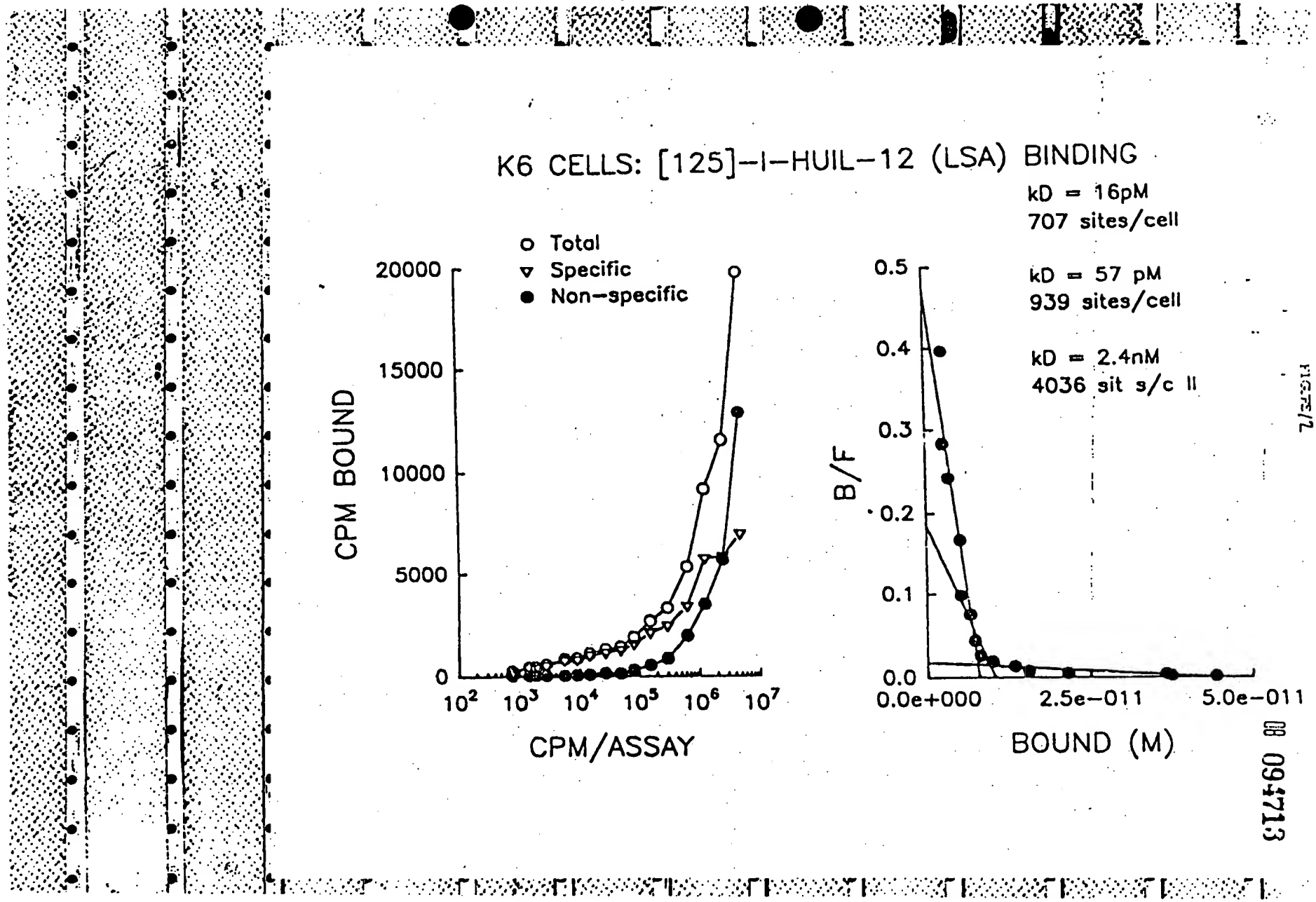
EP 0 094 713 B1

FIGURE 11

08 094713

125-I-24E6 COMPETITIVE BINDING TO K6 CELLS




K6 CELLS: [125]-I-HUIL-12 (LSA) BINDING
kD = 16pM
707 sites/cell
kD = 57 pM
939 sites/cell
kD = 2.4nM
4036 sit s/c II
Total
Specific
Non-specific
CPM BOUND
20000
15000
10000
5000
0
10^2 10^3 10^4 10^5 10^6 10^7
CPM/ASSAY
B/F
0.5
0.4
0.3
0.2
0.1
0.0
0.0e+000
2.5e-011
5.0e-011
BOUND (M)

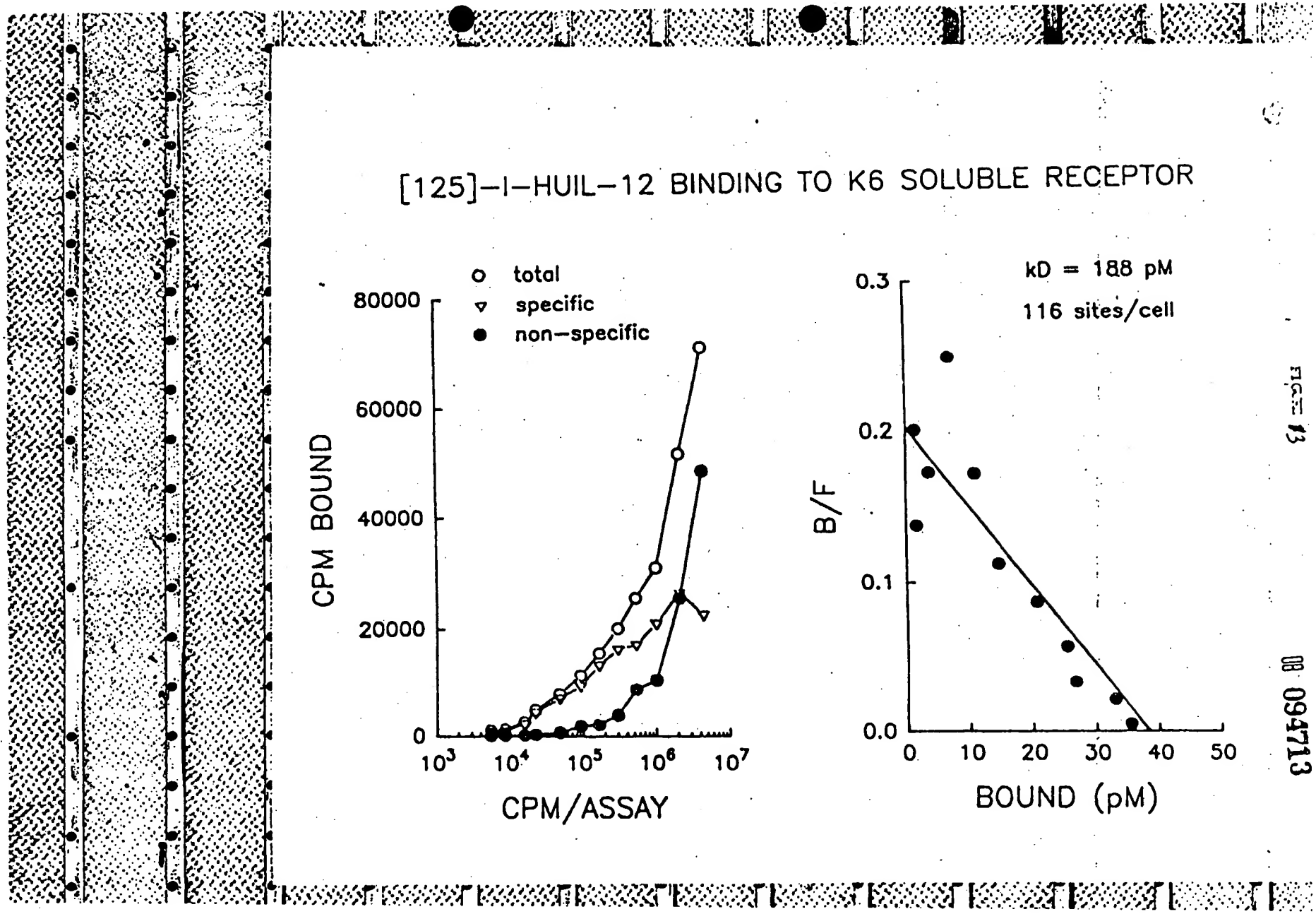


FIGURE 13

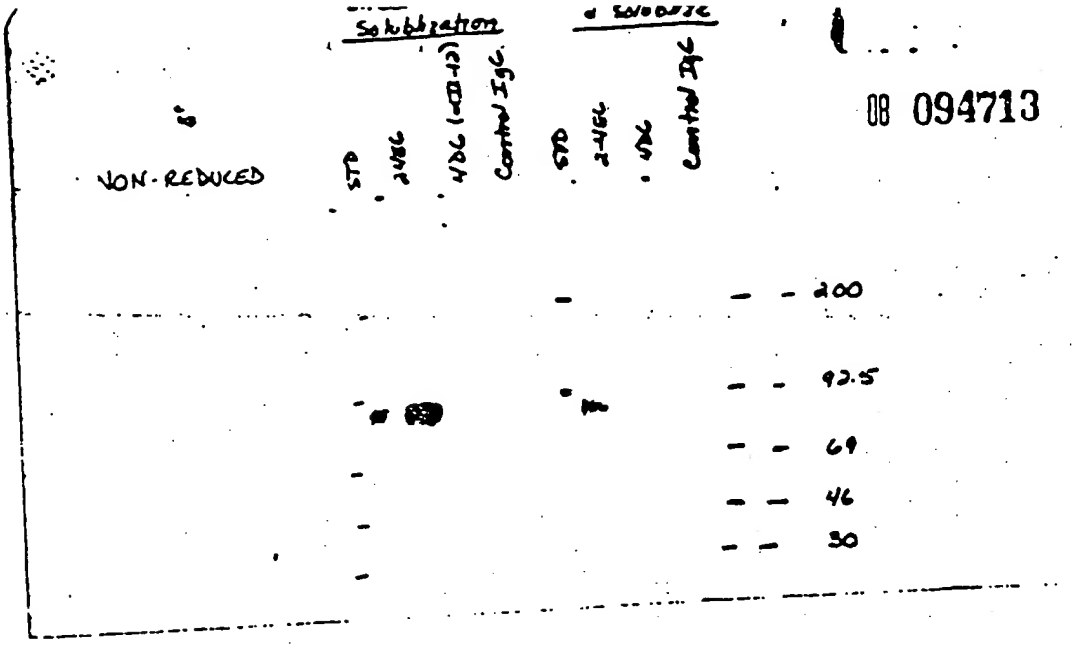
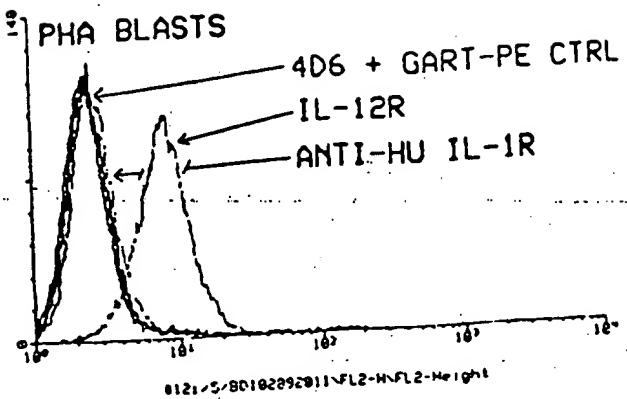
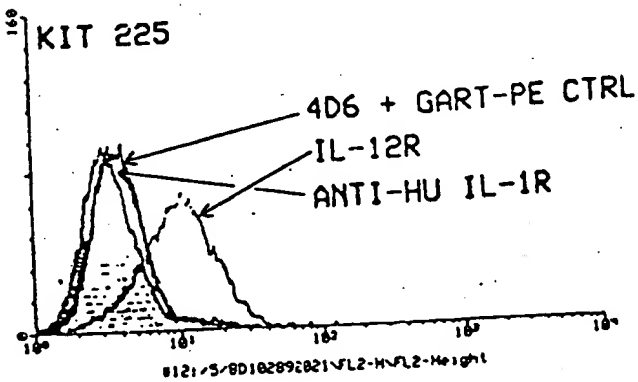
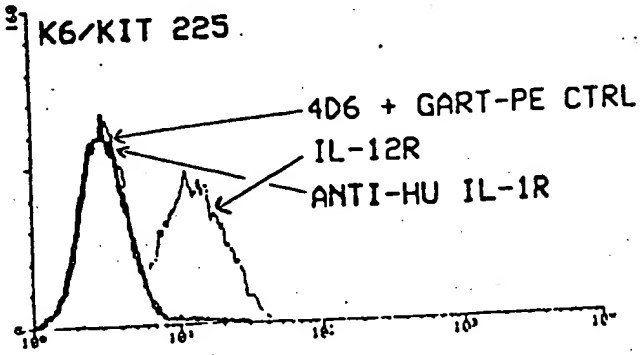

Solubilization
a Solvolysis
NON-REDUCED
STD
2-ME
4DG (-CB-13)
Control IgG
STD
2-ME
4DG
Control IgG
200
92.5
69
46
30

FIGURE 14


PHA BLASTS
4D6 + GART-PE CTRL
IL-12R
ANTI-HU IL-1R


KIT 225
4D6 + GART-PE CTRL
IL-12R
ANTI-HU IL-1R


K6/KIT 225
4D6 + GART-PE CTRL
IL-12R
ANTI-HU IL-1R